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APPENDIX 3

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REVIEW ARTICLE

Sequence Alignment of the G-Protein Coupled Receptor Superfamily

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ABSTRACT

The multitude of G-protein coupled receptor (GPR) superfamily cDNAs recently isolated has exceeded the number of receptor subtypes anticipated by pharmacological studies. Analysis of the sequence similarities and unique features of the members of this family is valuable for designing strategies to isolate related cDNAs, for developing hypotheses concerning substrate-ligand and receptor-effector interactions, and for undertanding the evolution of these genes. We have compiled and aligned the 74 unique amino acid sequences published to date and review the present understanding of the structural motifs contributing to ligand binding and G-protein coupling.

INTRODUCTION

THE CLONING of a great number of receptors and chan-I nels has revealed that many of these critical membrane proteins can be grouped into gene superfamilies based on sequence and structural similarities. One of these superfamilies comprises the G-protein coupled receptors (GPRs). Although the signal transduction mechanism is not known for all members of the gene family, in most cases receptor stimulation induces activation of a guanine nucleotide binding protein or G-protein. In 1982 the complete protein sequence of the visual pigment bovine rhodopsin was determined (Ovchinnikov et al., 1982). Its predicted structure; containing an extracellular amino terminus and seven hydrophobic membrane spanning α -helices (Hargrave et al., 1983), was remarkably similar to that previously identified by electron diffraction and sequence analysis for bacteriorhodopsin (Unwin and Henderson, 1975; Engelman et al., 1980). The subsequent molecular cloning of four human opsins (Nathans and Hogness, 1984; Nathans et al., 1986) and the hamster β -adrenergic receptor (Dixon et al., 1986) again revealed these structural features that have become the hallmark of this gene family (Fig. 1).

The number of GPRs that have been cloned is increasing rapidly; at present 74 distinct GPR sequences have been published. GPR cloning has led to the stable high-level expression of these receptor subtypes in mammalian cell lines, a preparation that has greatly aided the pharmacological characterization of these receptors. Molecular biological alteration of receptor sequences and expression in cell lines has provided much of our knowledge concerning the functional role of particular receptor regions and residues.

We have aligned all the available amino acid sequences of the members of this family (Fig. 2). This compilation should prove useful for designing cloning strategies for other GPRs. Indeed, many GPRs, among them the dopamine receptors (Bunzow et al., 1988; Dearry et al., 1990), the adenosine receptors (Libert et al., 1989b), and the cannabinoid receptor (Matsuda et al., 1990), have been cloned via approaches relying on sequence similarity. In addition, this sequence alignment may facilitate the formulation of hypotheses concerning the role of certain protein sequences in determining ligand binding, regulation, and G-protein specificity of the receptors. Comparison of the structure of the genes for these-receptors can provide insight into the evolution of this gene family.

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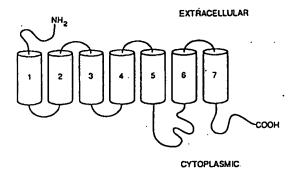


FIG. 1. The topography of G-protein linked receptors. Cylinders represent transmembrane α -helices. Extracellular and cytoplasmic sides of the plasma membrane are indicated.

The sequences were aligned manually, relying on invariate residues and published computer-generated sequence alignments. Several of the sequences, such as the FC5R receptor, have not yet been proven to represent GPRs. They are included in the alignment, however, because their sequences identify them as members of this superfamily. If sequences for the same receptor subtype of more than one species have been published, we have included only the sequence of the highest species. The sequences are organized into subgroups based on ligand type, i.e., muscarinic receptors, catecholamine receptors, etc.

GENERAL STRUCTURAL FEATURES

All of the proteins are single polypeptide chains. The shortest sequence represents the rat mas oncogene (324 amino acids) and the longest sequence represents the human thyroid-stimulating hormone receptor (744 amino acids). The predicted protein structures contain seven stretches of 20-30 hydrophobic amino acids which are believed to form membrane-spanning α -helices. These helices are referred to as transmembrane domains 1-7 (TM 1-TM 7). This predicted structure, based on hydropathy analysis, has been supported by electron diffraction analysis for bacteriorhodopsin (Henderson $et\ al.$, 1990) and proteolytic cleavage studies for rhodopsin and the β_1 -adrenergic receptor (Hargrave $et\ al.$, 1982; Dohlman $et\ al.$, 1988). The proteins have extracellular amino termini and cytoplasmic carboxyl termini.

The areas of greatest homology among the GPRs are in the seven transmembrane regions. Some residues are found in virtually all GPRs and may mediate the tertiary structure required for functional activity (Hulme et al., 1990; Hibert et al., 1991). Particularly well conserved are several proline residues in TM 4, 5, 6, and 7. These residues most likely introduce kinks in the α -helices and may be important in the formation of the binding pocket (Applebury and Hargrave, 1986; Findlay and Eliopoulos, 1990; Dahl et al., 1991; Hibert et al., 1991). Other well-conserved residues include a glycine, an asparagine, and a valine in TM 1; a leucine, two alanines, and an aspartate in TM 2; an isoleucine in TM3; a tryptophan in TM 4; a phenylalanine

and a tryptophan in TM 6; and an asparagine and a tyrosine in TM 7 (see Fig. 3). Certain conserved residues are replaced in particular subfamilies. For example, the TM 6 conserved tryptophan is replaced by methionine in the glycoprotein hormone receptors (Fig. 2).

Most GPRs have single conserved cysteine residues in each of the first two extracellular loops that are believed to form a disulfide bond that stabilizes the functional protein structure (see Fig. 3). Mutation of either of these conserved cysteine residues markedly alters the function of rhodopsin, muscarinic, and β -adrenergic receptors (Dixon et al., 1987a; Karnik et al., 1988; Fraser, 1989; Hulme et al., 1990). The most highly conserved intracellular sequence is the aspartate-arginine-tyrosine triplet adjacent to TM 3 which has been implicated in signal transduction (see below). The arginine of this triplet is invariant, and the aspartate and tyrosine are conservatively replaced in several GPRs.

The amino termini of these proteins vary greatly in length. They range from as few as seven residues in the adenosine A₂ receptor to over 300 residues for the glycoprotein hormone receptors. Overall, there is little sequence homology among the receptors in the first extracellular domain. The amino termini of nearly all the GPRs contain consensus sequences (N-X-S/T) for N-glycosylation (Kornfeld and Kornfeld, 1985). Rhodopsin, the α2-adrenergic, the β_1 -adrenergic, and the β_2 -adrenergic receptors are all glycosylated at several of these sites (Hargrave, 1977; Strasser et al., 1984; Benovic et al., 1987b; Dohlman et al., 1987; Regan, 1988). Glycosylation may contribute to the proper expression of membrane proteins (for review, see Kornfeld and Kornfeld, 1985). Deletion of the glycosylated domains of the β_2 -adrenergic receptor decreased the level of receptor expression but did not alter ligand binding (Dixon et al., 1987b). Inhibition of glycosylation diminished muscarinic receptor expression (Liles and Nathanson, 1986). The thyroid-stimulating hormone receptor contains six potential glycosylation sites. Mutational analysis demonstrated that two of these sites are required for the expression of functional receptor (Russo et al., 1991). Some receptors with short amino termini (the A₁ and A₂ adenosine and the α_{2B} -adrenergic receptors) do not contain amino-terminal asparagine glycosylation sites.

Phosphorylation and palmitoylation of carboxy-terminal sites can influence the signal transduction of some GPRs. Most GPRs contain potential phosphorylation sites in the third cytoplasmic loop and/or carboxyl terminus. For several receptors, phosphorylation by protein kinase A and specific receptor kinases mediates receptor desensitization (see Intracellular Coupling below, for discussion). Two adjacent cysteine residues in the carboxyl terminus of rhodopsin and one in the β_2 -adrenergic receptor are palmitoylated (Ovchinnikov et al., 1988; O'Dowd et al., 1989a). The hydrophobicity profile of the GPRs predict seven TM domains and thus three intracellular loops (Fig. 1). The palmitate on carboxy-terminal cysteine(s) would be expected to insert into the membrane, thereby forming an additional cytosolic loop which may influence receptor mobility (Findlay and Eliopoulos, 1990) or G-protein coupling (O'Dowd et al., 1989a).

FIG. 2. Amino acid sequence alignment of the GPR superfamily. The putative transmembrane domains are enclosed in boxes. The precise boundaries of the TM domains are not known with certainty. Dashes have been introduced for the purpose of alignment. Amino acids omitted from nonconserved regions are indicated by numbers in parentheses.

```
Dictyostelium cAMP receptor (Klein et al, 1988)
          Dog adenosine A2 receptor (RDC8) (Libert et al., 1989b)
Dog adenosine A1 receptor (RDC7) (Libert et al., 1989b)
 3.
          Human ml muscarinic acetylcholine receptor (Peralta et al., 1987)
          Human m2 muscarinic acetylcholine receptor (Peralta et al., 1987)
Human m3 muscarinic acetylcholine receptor (Peralta et al., 1987)
 6.
          Human m4 muscarinic acetylcholine receptor (Peralta et al., 1987)
          Human m5 muscarinic acetylcholine receptor (Bonner et al., 1988)
 8.
          Human beta 1 adrenergic receptor (Frielle et al., 1987)
Human beta 2 adrenergic receptor (Kobilka et al., 1987a)
Human beta 3 adrenergic receptor (Emorine et al., 1989)
10.
11.
          Cow alpha 1 adrenergic receptor (Schwinn et al., 1990)
12.
13.
          Rat alpha 1B adrenergic receptor (Voigt, et al., 1990)
          Human alpha 2 C4 adrenergic receptor (Regan et al., 1988)
          Human alpha 2 C2 adrenergic receptor (Lomasney et al., 1990)
Human alpha 2 C10 adrenergic receptor (Kobilka et al., 1987c)
15.
16.
          Human alpha 2 C10 adrenergic receptor (Kobilka et al.,
          Rat alpha 2 adrenergic receptor R20 (Lanier et al.,
17.
                                                                                       1991)
18.
          Drosophila octopamine receptor (Arakawa et al., 1990)
          Human dopamine D1 receptor (Dearry et al., 1990)
Human dopamine D5 receptor (Sunahara et al., 1991)
Human dopamine D2 receptor (Grandy et al., 1989)
Human dopamine D3 receptor (Giros et al., 1990)
19.
20.
21.
22.
23.
          Human dopamine D4 receptor (Van Tol et al., 1991)
          Human serotonin 1d receptor [RDC4] ( Hamblin and Metcalf, 1991)
Human serotonin 1a receptor (Kobilka et al., 1987b)
24.
          Rat serotonin 1c receptor (Julius et al., 1988)
Rat serotonin 2 receptor (Julius et al., 1990)
26.
27.
28.
          Human histamine H2 receptor (Gantz et al., 1991)
          Human N-formyl peptide receptor (Boulay et al., 1990)
Human C5a anaphylatoxin receptor.(Gerard and Gerard, 1991)
29.
30.
          Human thrombin receptor (Yu et al., 1991)
Human thromboxane A2 receptor (Hirata et al., 1991)
31.
32.
          Human IL-8 receptor (Murphy and Tiffany, 1991)
          Guinea-pig platelet-activating factor receptor (Honda et al, 1991)
          Cow endothelin 1 receptor (Arai et al., 1990)
36,
          Rat non-isopeptide selective endothelin receptor (Sakurai et al., 1990)
37.
          Mouse bombesin/gastrin releasing peptide receptor (Spindel et al., 1991)
         Rat neuromedin B preferring bombesin receptor (Wada et al., 1991)
Human vasoactive intestinal peptide (Szeedharan et al., 1991)
Rat neurotensin receptor (Tanaka et al., 1990)
Rat bradykinin receptor (McZachern et al., 1991)
38.
39.
40.
41.
          Mouse thyrotropin-releasing hormone receptor (Straub et al., 1990)
43.
          Human neurokinin A (SK) receptor (Gerard et al., 1990)
          Rat substance P receptor (Yokota et al., 1989)
          Rat neuromedin K receptor (Shigemoto et al., 1990)
Bovine adrenal angiotensin II type-1 receptor (Sasaki et al. 1991)
45
46.
          Human mas oncogene (angiotensin) receptor (Young et al., 1986)
47.
          Human lutropin-choriogonadotropin receptor (Frazier et al., 1990)
Human thyrotropin receptor (Libert et al., 1989a)
Human follicle stimulating hormone receptor (Minegish et al., 1991)
48.
49.
50.
51.
          Human rhodopsin (Nathans and Hogness, 1984)
          Human green opsin (Nathans et al., 1986)
52.
          Human red opsin (Nathans et al., 1986)
53.
          Human blue opsin (Nathans et al., 1986)
55.
          Odorant receptor F3 (Buck and Axel, 1991)
          Odorant receptor F5 (Buck and Axel, 1991)
Odorant receptor F6 (Buck and Axel, 1991)
Odorant receptor F12 (Buck and Axel, 1991)
56.
57.
58.
         Odorant receptor I3 (Buck and Axel, 1991)
Odorant receptor I7 (Buck and Axel, 1991)
59.
60.
          Odorant receptor IB (Buck and Axel, 1991)
Odorant receptor I9 (Buck and Axel, 1991)
61.
62.
          Odorant receptor I14 (Buck and Axel, 1991)
63.
          Odorant receptor I15: (Buck and Axel, 1991)
64.
          Human cannabinoid receptor (Matsuda et al., 1990)
          Mouse Glucocorticold-induced receptor (Harrigan et al., 1991)
67.
          Rat FC5R (Eva et al., 1990)
          Human endothelial cell GPR (Hla and Maciag, 1990)
68.
69.
          Rat testis G-protein coupled receptor 1 (Meyerhof et al. 1991a)
         Rat RGHJP (Meyerhof, DNA and Cell Biology, in press, 1991b). Human thoracic aorta GPR (Ross et al., 1990)
Cytomegalovirus (Human) GPR, US33 (Chee et al., 1990)
Cytomegalovirus (Human) GPR, US27 (Chee et al., 1990)
Cytomegalovirus (Human) GPR, US28 (Chee et al., 1990)
70.
71.
72.
73.
74.
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1 2	MGLLOGNPANET MSTMGSW
3	MPPAISAFQA
4 5 6 7 8	Mytsappavspnitvlapgkgpwq Mynstnssnnslaltspyktfe Mtlhnnsttssplfpnissswihspsdaglppgtvthfgsynvsraagnfssndgttddplgghtvwq Manftpvngssgngsvrlvtssshnryetve Megdsyhnattvngtpvnhqplerhrlwe
9	mcagvlvlcasepgnlssaaplpdcaataarllvpasppasllppasespeplsqqw
10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28	MAPWPHENSSLAPWPDLPTLAPNTANTSGLPGVPWE MGQPGNGSAF LIAPNRSHAPDHDVTQQRDEW MFLSGNASDSSNCTHPPPPPVNI SK MNPDLDTGHNTSAPAHWGELKDDNFTGPNQTSSNSTLPQLDVTR MASPALAAALAVAAAAGPNASGAGERGSGGVANASGASWGPPRGQYSAGA MDHQDPYSVQA MGSLQPDAGNASWNGTEAPGGGARATPYSLQV MGSLQPDAGNASWNGTEAPGGGARATPYSLQV MGSLQPDAGNASWNGTEAPGGGARATPYSLQV MGSLQPDAGNSSWNGTEAPGGGARATPYSLQV MGSLQPDAGNSSWNGTEAPGGGARATPYSLQV MGSLQPDAGNSSWNGTEAPGGGARATPYSLQV MGSLQPDAGNSSWNGTEAPGGGARATPYSLQV MGSLQPDAGNSSWNGTEAPGGGARATPYSLQV MGSLQPDAGNSSWNGTEAPGGGARATPYSLQV MGSLQPDAGNSWNGTEAPGGGARATPYSLQV MFLNISANDGTGLVVERDFSV MFLNISANDGTGLVVERDFSV MLPPGSNGTAYPGQFALYQOLAQGNAVGGSAGAPPLGPS MGPLNLSWYDDDLERQNWSRPFNGSDGKADRPH MASLSQLSSHLNSTCGAENSTGASQARPH MGNRSTADADGLLAGRGPAAGASAGASAGLACQ MSPLNQSAGCLPQCASNRSLNATETSEAWNPRTLQAL MDVLSPGQGNNTTSPPAPFETGGNTTGISDVTVSYQ MVNLGNAVSLLMHIGLLWGFDISI SPVAGIVTDTFNSSDGGRLFQFPDGV MEILCEDNISLSSIPNSIMQLGDGPRLYHNDFNSRDANTSEASNWTIDAENRTNLSCEGYLPPTCLSILHLQE MAPNGTASSFCLDSTACK
29 30 31 32 33	METNSSLPTNISGGTPAVSAGYLFLD MNSFNYTTPDYGHYDDKDTLDLNTPVDKTSNTLRVP MGPRRLLLVAACFSLCGPLLSARTRARRPESKATNATLDPRSFLLLRNPNDKYEPFWEDEEKNESGLTEYRLVSINKSSPLQKQLPAFISEDASGYLTSSWL MWPNGSSLGPCFRPTNITLEERR MESDSFEDFWKGEDLSNYSYSSTLPPFLLDAAPCEPESLEIN
34	MELNSSSRVDSEFRYT
35	metfwlrlsfwvalvggvisdnpesystnlsihvdsvatfhgtelsfvvtthoptnlalpsngsminycpootkitsafk
36	MQSSASRCGRALVALLLACGLLGVWGEKRGFPPAQATPSLLGTKEVMTPPTKTSWTRGSNSSLMRFRTAEVTKGGRVAGVPPRSFPPPCQRK1EINKTFK
37 38	MMAPNNCSHLNLDVDPFLSCNDTFNQSLSPP(n/DNWFHPGF
39	MPPRSIPILSUFTEASESELEPECWENDFLPOSDGTTAELVIR
40	MDLHLFDYAEPGNFSDLSWPCNSSDCIVVDTVMCPNMPNKSVLL MHLNSSVPQGTPGEPDAQPFSGPQSEMEATFLALSLSNGSGNTSESDTAGPNSDLDVNTDIYS
41	Manufacture of the control of the co
42	MENDITY SEMPOTE LOPOAAVALEY OVYT
43	MGTCDIVTEANISSGPESNTTGITAFSMPSWQ
44	MDN/LPMDsdlfpni stntsesnoff/optwo
45 46	MASVPRGENWTDGTVEVGTHTGNLSSALGVTEWIAIQAGNFSSALGLPATTQAPSQVRANLTNQFVQPSWR
47	MILNSSTEDGIKRIOODCPKAGRHNYIFI MDGSNVTSFVVEEPTNISTGRNASVGNAHROIP
400	WARREN TO THE TOTAL TOTAL TO THE TOTAL TO TH
48 49 50	MKQRFSPLQLLKLLLLLQAPLPRALRRLCPEPCN-(248)-LPTKELNFSHSISENFSKQCESTVRKSELSGADYEYGFCLPKTPRCAPEPDAFNPCEDIMG MRPADLLQLVLLLDLPROLGGMGCSSPPCECHQE-(318)-YVFFEEQEDEIIGFGQELKNPQEETLQAFDSHYDYTICGDSEDMVCTPKSDEFNPCEDIMG MALLLVSLLAFLSLGSGCHHRICHCSNRVFLCQE-(266)-VDYMTQARGQRSSLAEDNESSYSRGFDMTYTEFDYDLCNEVVDVTCSPKPDAFNPCEDIMG
51	MNGTEGPNFYVPFSNATGVVRSPFEYPQYYLAEPWQF
.52	MAQQNSLQRLAGRHPQDSYEDSTQSSIFTYTNSNSTRGPFEGPNYHIAPRWYYHLTSVW
53 54	MAQOWSLORLAGRHPQDSYEDSTQSSIFTYTNSNSTRGPFEGPNYHIAPRWYYHLTSVW
JN	MRVMSEZEFYLFKNISSVGPWDGPQYHAIPVWAFYL
55	MDSSNRTRVSEFLILGFVENKDLOP
56	MSSTNQSSVTEFLLIGLSRQPQQQQ
57 58	MAWSTGONLSTPGPFI LLGFPGPRSMRI
59	MESGNSTRRFSSFFLLGFTENPQLHF
60	MNNQTFITQFLLIGLPIPEEHQH MERRNHSGRVSEFVLIGLPAPAPLRV
ត	MINITY I THE LEFT PER HOUSE TO THE MINITY I THE LEFT PER HOUSE THE PER H
62	MTRRNOTAL SOFFLIGLPFPPEYOH
63	: MTGNNOTLILEFLLLGLPIPSEYHL
.64	MTEENQTVISQFLLLFLP1PSEHQH
65	MKSILDGLADTTFRTITTDLLYVGSNDIQYEDIK-(21)-SPFQEKMTAGDNSPLVPAGDTTNITEFYNKSLSSFKENEENIQCGENFMDMECFMILNPSQQ
66	kvppvlllfllssvrateqpqvvtehpsweaaltgpnasshfwanytfsdwqnfvgrrrygaesqnptv
ត	MNSTLSFRVENYSVHYNVSENSPFLAFENDDCHLPLAV
68 69	MCPTSVPI.VKAHRSSVSDYVNYDI IVRHYNYTGKLNI SADKENS IK
70	MKANNITTSALMLQ MFPNGTAPSPTSSPSSSPGGGGGGVCSRGPGSGAADGMEEPGRNSSONGTLSEGOGS
71.	MAGNCSWEAHSTNON: MCCOMSEALELY SRGFLT I EQI ATLPPPA
72	MICPLEAR
73	MITSTNNOTLTOVSNMTNHTLNSTE I YOLFEYTR
74	MIPTITAELITEFDYDEDATPCVFTDVLWQSK

1	SLVLLLFADFSSMLGCMAVLI	GFWRLKLLRNHVTK	-VIACFCATSFCKDFPSTILTLT-	NTAVNGGFPCYLYA
2	VYITVELAIAVLAILGNVLVCWAV-	WLNSNLONVIN .	-YFVVSLAAADIAVGVLAIPFAIT	ISTGFCAACHN CL
3	AYIGIEVLIALVSVPGNVLVIWAV-	KVNQALRDATF	-CFIVSLAVADVAVGALVIPLAIL	
				YI.IMGH-WALGTIACD
4	VAFIGITTGLLSLATVTGNLLVLISF-	KVNTELKTVNN	-YFLLSLACADLIIGTFSMNLYTT	YLIMGH-WALGTIACDYTVIGY-WPLGPVVCD
5	VVFIVLVAGSLSLVTIIGNILVMVSI-	KVNRHLQTVNN	-YFLFSLACADLIIGVFSMNLYTL	YI IMNR-WALGNIACD
6	VVFIAFLTGILALVTIIGNILVIVSF-	KVNKQLKTVNN	-YFLLSLACADLIIGVISMNLFTT -YFLFSLACADLIIGAFSMNLYTV	YIIKGY-WPLGAVVCD
7	MVFIATVTGSLSLVTVVGNILVMLSI-	KVNRQLQTVNN KVNSQLKTVNN	-YYLLSIACADLIIGIFSMNLYTT	YILMGR-WALGSLACD
8	VITIAVVTAVVSLMTIVGNVLVMISF-		-11msiAcabatton binatti	
9	TAGMGLIMALIVLLIVAGNVLVIVAIA	KTPR-LQTLTN	-LFIMSLASADLVMGLLVVPFGAT	LVVWGR-WEYGSFFCE
10	VVGMGIVMSLIVLAIVFGNVLVITAIA	KFER-LOTVIN	-YFITSLACADLVMGLAVVPFGAA	
11	AALAGALIALAVLATVGGNLLVIVAIA	WTPR-LOIMIN	-VFVTSLAAADLVMGLLVVPPAAT	LALTGH-WPLGATGCE
12	AILLGVILGGLILFGVLGNILVILSVA	CHRHLHSVTH	-YYIVNLAVADLLLTSTVLPFSAI.	FEILGY-WAFGRVFCN
13	AISVGLVLGAFILFAIVGNILVILSVA	CNRHLRTPTN	-YFIVNLAIADLLLSFTVLPFSAT	LEVIGY-WVLGRIFCD
14	VAGLAAVVGFLIVFTVVGNVLVVIAVL	TSRALRAPON	-lflvslasadilvatlvmpfsla	NELMAY-WYFGQVWCG
15	TAAIAAAITFLILFTIFGNALVILAVL	TSRSLRAPQN	-LFLVSLAAADILVATLIIPFSLA	NELLGY-WYFRRTWCE
16 [.]	TLTLVCLAGLIMLLTVFGNVLVIIAVF	TSRALKAPON	-LFLVSLASADILVATLVIPFSLA	NEVNGY-WYFGKTWCE
17	TLTLVCLAGLIMLFTVFGNVLVIIAVF	TSRALKAPON	-LFLVSLASADILVATLVIPFSLA	
18	LLTALVLSVIIVL-TIIGNILVILSVF	TYKPLRIVON	-FFIVSLAVADLTVALLVLPFNVA	YSILGR-WEFGIHLCKAEIAGF-WPFGSFCN
19 ·	RILTACFLSLLILSTLLGNTLVCAAVI	RFRHLRSKVIN	-FFVISLAVSDLLVAVLVMPWKAV -VFIVSLAVSDLFVALLVMPWKAY	
20	QVVTACLLTLLI IWTLLGNVLVCAAIV	RSRHLRANMIN	-YLIVSLAVADLLVATLVMPWVVY	LEVVGE-WKFSRIHCD
21	YNYYATLLTLLIAVIVFGNVLVCMAVS	REKALQTTTNREKALQTTTN	-YLVVSLAVADLLVATLVMPWVVY	LEVTGGVWNFSRICCD
22 23	-AYYALSYCALILAIVFGNGLVCMAVL GAAALVGGVLLIGAVLAGNSLVCVSVA	TERALOTPIN	-SFIVSLAAADLLLALLVLPLFVY	SEVQGAAWLLSPRICD
23 24	KISLAVVLSVITLATVLSNAFVLTTIL	LTRKLHTPAN	-YLIGSLATIDLLVSILVMPISIA	YTITHT-WNFGQILCD
25	VITSLLIGTLIFC-AVLGNACVVAAIA	LERSLONVAN	-YLIGSLAVIDLMVSVLVLPMAAL	YQVLNK-WTLGQVTCD
26	QNWPALSIVVIIINTIGGNILVIMAVS	MEKKLHNATN	-YFIMSLAIADMLVGFLVMPLSLL	
27	KNWSALLTTVVIILTIAGNILVIMAVS	LEKKLONATN	-YFIMSLAIADMLLGFLVMPVSML	TILYGYRWPLPSKLCA
28	-ITITVVLAVLILITVAGNVVVCLAVG	LNRRLRNLTN	-CFIVSLAITDLLIGLLVLPFSAI	YQLSCK-WSFGKVFCN
29	-IITYLVFAVTFVLGVLGNGLVIWVAG	FRMTHTVTT	-ISYLNLAVADFCFTSTLPFFMVR	KAMGGHWPFGWFLCK
30	DILALVIFAVVFLVGVLGNALVVWVTA	FEAKRTINA-	-IWFLNLAVADFLSCLALPILFTS	IVQHHHWPFGGAACS
31	TLFVPSVYTGVFVVSLPLNIMAIVVFI	LKMKVKKPAV	-VYMLHLATADVLFVSVLPFKISY	YFSGSDWQFGSELCR
32.	-YINTVISCTIFIVOMVGNATLLRIIY	ONKCMRNGPN	-ALIASLALGDLIYVVIDLPINVP	KLIAGRWPFEQNDFGVFICK
33	KYFVVIIYALVFLLSLLGNSLVMLVIL	YSRGVRSVID	-VYLLNLALADLLFALTLPIWAAS -IFMVNLTVADLLFLITLPLWIVY	KVNGWIFGTFLCK
34	LFPIVYSIIFVLGIIANGYVLWVFA	RLYPSKKNEIK-	-ILIASLALGOLLHIIIDIPINAY	KLLAGDWPFGAEMCK
35 36	-YINTIVSCLVFVLGIIGNSTLLRIIY LIASPWFAASFCVVGLASNLLALSVLA	GARQSSSHTRSSFL	-TFLCGLVLTDFLGLLVTGTIVVS	
36 37	IYVIPAVYGLIIVIGLIGNITLIKIF-	CTVKSMRNVPN	-LFISSIALGDLLLLVTCAPVDAS	KYLADRWLFGRIGCK
38	CVIPSSLYLIII5VGLLGNIMLVKIF-	LTNSTMRSVPN	-IFISNLAAGDLLLLLTCVPVDAS	
39	-YTLSFIYIFIFVICMIANSVVVWVNI	QAKTTGYDTH	-CYILNLA IADLWVVLTIPVWVVS	
40	KVLVTAIYLALFVVGTVGNSVTAFTLA	RKKSLQSLQSTVH-	-YHLGSLALSDLLILLLAMPVELY	NFIWVHHPWAFGDAGCR
41	AIQAPFLW-VLFLLAALENIFVLSVFC	LHKTNCTVAE	-iylgnlasadlilacglpfwäit	CR
42	-ILLVVIICGLGIVGNIMVVLVVM	RTKHMRTPTN .	-CYLVSLAVADLMVLVAAGLPNIT	DSIYGS-WYGYVGCL
43	LALWATAYLALVLVAVTGNAIVIWIIL	AHRRMRTVIN	-YFIVNLALADLCHAAFNAAFNFV	YASHNIWYFGRAFCY
44	IVLWAAAYTVIVVTSVVGNVVVIWIIL	AHKRMRTVTN	-YFLVNLAFAEACMAAFNTVVNFT	YAVHNVWYYGLFYCKYGLHSEWYFGANYCR
45	IALWSLAYGLVVAVAVFGNLIVIWIIL	AHKRMRTVTN	-YFLVNLAFSDASVAAFNTLINFI	TAMEYRWPFGNYLCK
46	MIPTLYSIIFVVGIFGNSLVVIVIYIVHWVIMSISPVGFVENGILLWFLC	FYMKLKTYAS	-VFLLNLALADLCFLLTLPLWAVY -TVYTHLSIADISLLFCIFILSID	YALDYELSSGHYYTIV
47	I VHWV IMSISPVGF VERGIEDWFIX	FRMRRNPF	-IVII ALSIADISMI CITILISID	
48	YDFLRVLIWLINILAIMGNVMTLFVLL	TSRYKLTVPR	-FIMCNLSFADFCMGLYLLLIASV	DSQTKGQYYNHAIDWQTGSGCS
49	YKFLRIVVWFVSLLALLGNVFVLLILL	TSHYKLNVPR	-FIMCNLAFADFCMGMYLLLIASV	DLYTHSEYYNHAIDWOTGPGCN
50	YNI LRVLIWFISILAITGNI IVLVILT	TSOYKLTVPR	-FIMENLAFADLCIGIYLLLIASV	DIHTKSQYHNYAIDWQTGAGCD
~		20,21122111		
51	SMLAAYM-FLLIVLGFPINFLTLYVTV	OHKKLRTPIN	-YILLNLAVADLFMVLGGFTSTLY	TSLHGYFVFGPTGCN
52	MIFVVIASVF-INGLVLAATM	KFKKLRHPLN	-wilvnlavadlagiviastisvv	CV
53	MIFVVTASVF-TNGLVLAATM	KFKKLRHPLN	-WILVNLAVADLAGTVIASTISIV	CV
54	QAAFM-GTVFLIGFPLNAMVLVATL	AYKKLROPIN	-YILVNVSFGGFLLCIFSVFPVFV	
				ing introductivity
55	-LIYGLFLSMYLVTVIGNISIIVAII	SDPCLHTPM-	YFFLSNLSFVDICFISTTVP	KVLCI KVLCL
56	LIFLIFLIMYLATVLGNLLIILAIG	GDSRLHTPM-	YFFLSNLSFVDVCFSSTTVP	KTLATFAPRGGVISLAGCA
57	GLFLLFLVMYLLTVVGNLAIISLVG	AHRCLOPMT-	YFFLCNLSFLEIWFTTACVP YFFLANLSFVDICFTSTTIP	MILVNIYTQSKSITYEDCI
S8	LIFALFLSMYLVTVLGNLLIIMAII	TOSHLHTPM-	YLFLSNLSFSDLCFSSVTMP	KLLQNMRSQDTSIPYGGCL
59	LIFFLSLLXYVLVLTENMLIIIAIR	IDSQLHTPM- NHPTLHKPM-	YFFLANMSFLEIWYVTVTIP	KLMAGFIGSKENHÖQLISFEACM
60 61	-LFFALFLIMYLTTFLGNLLIVVLVQ	LDSHLHTPM-	YLFLSNLSFSDLCFSSVIML	KLLQNIQSQVPSISYAGCL
62	-LFYALFLAMYLTTLLGNLIIILLL	LDSHLHTPM-	YLFLSNLSFADLCFSSVTMP	KLLCNMQSQVPSIPYAGCL
63	-LFYALFLAMYLTIILGNLLIIVLVR	LDSHLHMPM-	YLFLSNLSFSDLCFSSVIMP	KLLQNMQSQVPSISYTGCL
64	-VFYALFLSMYLTTVLGNLIIIILIH	LDSHLHTPM-	YLFLSNLSFSDLCFSSVTMP	KLLQNMQSQVPSIPFAGCL
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65	-LAIAVLSLTLGTFTVLENLLVLCVIL	HSRSLRCRPSY	-HFIGSLAVADLLGSVIFVYSFVD	FHVFHRKDSPNVFL
66	KALLIVAYSFTIVFSLFGNVLVCHYIF	KNORMISATS	-LFIVNLAVADIMITLLNTPFTLV	
67	IFTIALAYGAVIILGVSGNLALIIIIL	KOKEMRNVTN	-ILIVNLSFSDLLVAVMCLPFTFV	
68	-LTSVVFIL-ICCFIILENIFVLLTIW	KTKKFHRPMY	-YFIGNLALSDLLAGVAYTANLLL	SGATTYKLTPAQWF
69	ITYYITMEAAIGLCAVVGNMLVIWVV-	KLNRTLRTTTF-	-YFIVSLALADIAVGVLVIPLAIA	SAWRSRCTSMACL
70	AILISFIYSVVCLVGLCGNSMVIYVIL	RYAKMKTATN	-IYILNLAIADELIMLSVPFLVTS	
71	VTNYIFLLLCLCGLVGNGLVLWFFG	FSIKRTPFSIY	-IYFLHLASADGIYLFSKAVIALL	NOWLLPAGVASCK
72	-TTEAVLNTFIIFVGGPLNAIVLITQL	LTNRVLGY-STPT- YYRRKKKSPSD	-IYMTNLYSTNFLTLTVLPFIVLS -TYICNLAVADLLIVVGLPFFLEY	CS
73 74	LGVWLMCIVGTFLNVLVITTIL -PVTLFLYGVVFLFGSIGNFLVIFTIT	-WRRRIQCSGD	-VYFINLAAADLLFVCTLPLWMQY	CT
14	" ATTE DIGAAL DE GOTONE PATEITT.		· · · · · · · · · · · · · · · · · · ·	•

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1	IVITYGSFACHLWTLCLAISI)	MLIVKREPEPELFEK	-YYYLLCWGLPLISTIVMLA-	KNTVQFVGN
2	FFACEVLVLTQSSIFSLIAIAI		-AKGIIAVCWVLSFAIGLTPML-GW	NNCSOPKEGRNYSO
3	MVACPVLILTQSSILALIAIA	DRYLRVKIPLRYKTVVTPRR	-AAVAIAGCWILSFVVGLTPLF-GW	NRLGEAQRAWAANGSGGEPVI
4	IWLALDYVASNASVINILLISF	DRYFSVTRPLSYRAKRT-PRR		1
5	LWLALDYVVSNASVMVLLIISF			
6	LWLAIDYVASNASVLNLLVISF			
7	LWLALDYVVSNASVMNLLIISF		The state of the state of	QFVVGKRTVPDN
8	LWLALDYVASNASVLNLLVISF	DRYFSITRPLTYRAKRT-PKR		
9	LWTSVDVLCVTASIETLCVIAL	DDVI I VEGDONIACI I COLO		
10	FWTSIDVLCVTASIETLCVIAV			
11	LWTSVDVLCVTASIETLCALAV		-ARVIILMVWIVSGLTSFLPIQMHW -ARTAVVLVWVVSAAVSFAPIMSQW	YRATHQEAIWRVGADAEAQ
12	WAAVDVLCCTASIMGLCIISI		-GLMALLCVWALSLVISIGPLF-GW	
13	IWAAVDVLCCTASILSLCAIŠI	DRY IGVRY SLOYPTLYTRRX	-AILALLSVWVLSTVISIGPLL-GW	KEPAPNDDK
14	VYLALDVLFCTSSIVHLCAISL	DRYWSVTQAVEYNLKRTPRR	-VKATIVAVWLISAVISFPPLVSLY	
15	VYLALDVLFCTSSIVHLCAISL		-IKCIILTVWLIAAVISLPPLIYKG	DOGPOPRGRP
16 17	IYLALDVLFCTSSIVHLCAISL IYLALDVLFCTSSIVHLCAISL	***************************************	-IKAIIITVWVISAVISFPPLISI-	FKKCCCCCDODARD
18	LWLTCDVLCCTSSILNLCAIAL		-IKAIIVTVWVISAVISFPPLLISI	EKKGAGGGOOPAEP NDWPDEFTSAT
19	IWVAFDIMCSTASI LINLCVI SV		-VLLLISGVWLLSLLISSPPLI-GW	NDWPDEFTSAT
20	VWVAFDIMCSTASILNLCVI SV		-AFILISVAWTLSVLISFIPVQLSW -ALVMVGLAWTLSILISFIPVQLNW	HKAKPTSPSDGNATSLAETID
21	IFVTLDVMMCTASILNLCAISI	DRYTAVAMPMLYN-TRYSSKRR	-VIVMISIVWVLSFTISC-PLLFGL	NRDQAASWGGLDLPNN-(20)
22 ·	VFVTLDVMMCTASI LNLCAI SI	DRYTAVVMPVHYOHGTGOSSCRR	-VALMITAVWVLAFAVSC-PLLFGF	
23	ALMAMOVMICTASIFNICALSV	DRFVAVAVPLRYNROGGSRR	-QLLLIGATWLLSAAVAA-PVLCGL	
24	IWLSSDITCCTASILHLCVIAL	DRYWAITDALEYSKRRTAGH-	-AATMIAIVWAISICISIPPLFW	ROAKAOEEMS
25 26	LFIALDVLCCTSSILHLCAIAL VWISLDVLFSTASIMHLCAISL	DRYWAITOPIDYVNXRTPR	-PRALISLTWLIGFLISIPPM-LGW	
27	IWIYLDVLFSTASIMHLCAISL	DRYVAIRNPIEHSRF-SRTK DRYVAIQNPIHHSRFNSRTK	-AIMKIAIVWAISIGVSV-PIPVIG	LRDESKVFVNNT
28	IYTSLDVMLCTASI LNLFMI SL	DRYCAVMDPLRYPVLVTPVR	-AFLKIIAVWTISVGISM-PIPVFG	LQDDSKVFKEG
		SHI GIVI DE EKTEVEVITEVK	-VAISLVLIWVISITLSFLSIHLGW	NSRNETSKGNHTTS
29	FLFTIVDINLFGSVFLIALIAL	DRCVCVLHPVWTQNHRTVSLAK-	KVIIGPWVMALLL-TLPVII	RVTTVPGKTGTV
30 .	ILPSLILLNMYASILLLATISA	DRFLLVFKPIWCONFRGAGL	-AWIACAVAWGLALLL-TIPSFLY-	RVVREEYFPPKV
31 32	FVTAAFYCNMYASILLMIVISI	DRFLAVVYPMQSLSWRTLGR	-ASFTCLAIWALAIAG-V-PLVL	KEOTTOWICTNIT
33	FMGVVMIFFGLSPLLLGAAMAS VVSLLKEVNFYSGILLLACISV	ERYLGITRPFSRPAVASQRR	-AWATVGLVWAAALALGLLPLL-GV	GRYTVOYPGS
34	LAGCLFFINTYCSVAFLGVITY	DRYLAIVHATRTLTOKRHLVK— NRFQAVKYPIKTAQATTRKR——	FICLSIWGLSLLL-ALPVLL	FRRTVYSSNVSP
35	LFPFLQKSSVGITVLNLCALSV	DRYRAVASWSRVQGIGIPLV	-GIALSLVIWVAIVAA-ASYFLVMM -TAIEIVSIWILSFIL-AIPEAIGF	DSTNVVSNKAGSGNIT
36	LVPFIQKASVGITVLSLCALSI	DRYRAVASWSRIKGIGVPK	WTAVEIVLIWVSVVL-AVPEAIGF	
37	LIPFIQLTSVGVSVFTLTALSA	DRYKAIVRPMDIQASHALMK	-ICLKAALIWIVSMLL-AIPEAVF-	SDLHPFHVKDTNOTFI
38 [°]	LIPAIQLTSVGVSVPTLTALSA	DRYRAI VNPMDMQTSGVVI	-WISVAVGIWVVSVLL-AVPEAVF-	SEVARI-GSSDNSSFT
40	VTHLIFSINLFSGIFFLTCMSV GYYFLRDACTYATALNVASLSV	DRYLSITYFTNTPSSRKKMVRR-	AVCILVWLLAFCV-SLPDTYYL	KTVTSASNNET
41	VVNTMI YMNLYSSI CFLMLVSI	ERYLAICHPFKAKTLMSRSRTK- DRYLALVKTMSMGRMRGVR	KFISAIWLASALL-AIPMLFT-	MGLQNRSGDGTHPGGL
42	CITYLOYLGINASSCSITAFTI	ERYIAICHPI KAOFICTFSR	WAKLYSLVIWSCTLLL-SSPMLVFR	TMKDYREEGHNV
43	FONLFPITAMEVSIYSMTAIAA	DRYMAIVHPFQPRLSAPSTK	-AKKIIIFVWAFTSIYCMLWFFLLD AVIAGIWLVALAL-AFPQCFY-	LNISTYKNAVVV
44	PHNFFPIAALFASIYSMTAVAF	DRYMAI IHPLOPRLSATATK	WIFVIWVIALLL-ASPOGYY-	STVIMDOGATSTTETMPSRV
45	FONFFPITAVFASIYSM-AIAV	DRYMAIIDPLKPRLSATATK	IVIGSIWI LAFLL-AFPOCLY-	SKI KVMPGRT
46 47	IASASVSFNLYASVFLLTCLSI	DRYLAIVHPMKSRLRRTML	VAKVTCIIIWLLAGLA-SLPTIIHR	NEFTENTNIT
٠,	TLSVTF LFGYNTGLYLLTAI SV	ERCLSVLYPIWYRCHRPKY	QSALVCALLWALSCLVTTME-YVM-	CIDREEESHSRN
48	TAGFFTVLASELSVYTLTVITL	ERWHTITYAIHLDQKLRLRH	-ATTIMICON FORT TANGET IN	
49	TAGFFTVFASELSVYTLTVITL	ERWYAITFAMRLDRKIRLRH	-AILIMLGGWLFSSLIAMLPLVGVS -ACAIMVGGWVCCFLLALLPLVGIS	NYMKVS
50	AAGFFTVFASELSVYTLTAITL	ERWHTITHIMQLDCKVQLRH	-AASVMVMGWIFAFAAALFPIFGIS	SYAKVSSYMKVS
_	1 honny			
51 52	LEGFFATLGGE IALWSLVVLAI	ERYVVVCKPMSNFRFGEN	HAIMGVAFTWVMALA-CAAPPLAGW	SRY IPEGLOC
53	LEGYTVSLCGITGLWSLAIISW LEGYTVSLCGITGLWSLAIISW	ERWMVVCKPFGNVRFDAK	LAIVGIAFSWIWAAV-WTAPPIFGW	SRYWPHGLKT
54	LEGFLGTVAGLVTGWSLAFLAF	ERWLVVCKPFGNVRFDAK	LAIVGIAFSWIWSAV-WTAPPIFGW	
		ENTITION OF A SSK	HALTVVLATWTIGIG-VSIPPFFGW	
55	TOIYFFLLFVELDNFLLTIMAY	DRYVAICHPMHYTVIMNYK	LCGFLVLVSWIVSVLHALFQSIMML	ALPFCTHLEIPHY
56	TOLYFLAVFGNMDNFLLAVMSY	DRYVAICHPLHYTTKMTRQ	LCVLLVVGSWVVANMICLLHILLMA	RKSFCADNMIPHF
57	TOMYFVFSLGCTEYFLLAVMAY	DRYLAICLPLRYGGIMTPG	LAMRLALGSWLCGFSAITVPATLIA	
58 59	SOMCVFLVFAELGNFLLAVMAY AQTYFFMVFGDMESFLLVAMAY	DRYVAXCHPLCYTVIVNHR	LCILLLLSWVISIFHAFIQSLIVL	QLTFCGDVKIPHF
60	TOLYFFIGLECTECVLLAVMAY	DRYVAICFLPHYTSIMSPK	LCTCLVLLLAMLTTSHAMMITLLAA	RLSFCENNVVLNF
61	TOIFFFLLFGYLGNFLLVAMAY	DRYVAICHPLHYPVIVSSR DRYVAICFPLHYTNIMSHK	LCVOMAAGSWAGGFGISMVKVFLIS	RLSYCGPNTINHF
62	AQIYFFLFFGDLGNFLLVAMAY	DRYVAICFPLHYMSIMSPK	LCTCLLLVFWIMTSSHAMMHTLLAA LCYSLVVLSWVLTTFHAMLHTLLMA	RLSFCENNVILINF
63	TOLYFFMVFGDMESFLLVVMAY	DRYVAICFPLRYTTIMSTK	FCASLVLLLWMLTMTHALLHTLLIA	
64	TOLYFYLYFADLESFLLVAMAY	DRYVAICFPLHYMSIMSPK	LCVSLVVLSWVLTTFHAMLHTLLMA	RLSFCADNMIPHF
ය	FUI COUTA SETTACUCOS OS TO -	B.007-2-1-1-1		.aut Chiarphi
66 66	FKLGGVTASFTASVGSLFLTAI VSRFAQYCSLHVSALILTAIAV	DRYISIHRPLAYKRIVTRPK	-AVVAFCLMWTIAIVIAVLPLL-GW	NCKKLQS
ี ถึง	LNPFVQCVSITVSIFSLVLIAV	DRHQVIMHPLKPRISITKG ERHQLIINPRGWRPNNRH	VIYIAVIWVMATFF-SLPHAIC-	
68	LREGSMFVALSLSVFSLLAIAI	ERYITMLKMKLHNGSNNFR	-AYIGITVIWVLAVAS-SLPFVIY-	QILTDEPFONVSLAAFKDKY
69	FMSCVLLVFTHASIMSLLAIAV	DRYLRVKLTVRYRTVTTORR—	-LFILISACWVISLILGGLPIM-GW -IWLFLGLCWLVSFLVGLTPMF-GW	NCISALS .
70	LVLSVDAVNMFTSIYCLTVLSV		VAKVVNLGVWVLSLLV-ILPIVVFS	NRKVTLELSQNSSTL
71	VSRIVGLCTFFAGVSLLPAISI	ERCVSVIFPMWYWRRRPKR	LSAGVCALLWLLSFLV-TSIHNYF-	RTAANSDGTV
72			STYMILLLTWLAGLI-FSVPAAVYT	TVVMHDANDTNNTNGHA
-		DRYCVIVWGVELNRVRNNKR	ATCWVVIE-WILAVL-MGMPHYLMY	SHTNN
L	The state of the s	DRYYAIVYMRY——RPVK	QACLFSIFWWIFAVI-IAIPHFMVV	TKKDN

1 2			SRYTYVVIHNGVSDN
	WCWIGVSFTGYRFG	-LFYPFLFIWAISAVLVGLT-	RIFLAARROLKOMESOPLPGERARSTLQ EVFYLIRROLGKKVSASSGDPOKYYG
	GCGEGQVACLFEDVVPMV	YMVYYNF FAFVLVPLLLMLGVYL-	KIL TWKKOPKOLESOLPLODIVIO 195
3	KCEFEKVI SME	YMVYFNF FVWVLPPLLLMVLIYL-	EALIDIKE TOWNS AND SORT ALLES
ALESSON.			RIYRETENRARELAALQGSETPGKGGGSSSSSERSQPGAEGSPETP
4	QCYIQFLSQP	I ITFGTAMAAFYMPVTVMCTLYW-	HISRASKSRIKKOKKEPVANODPVSPSLVOGRIVKPNNNMPSSDD
5	ECYIOFFSNP	AVTFGTATAAFYLPVITMTVLYW-	RIYKETEKRIKELAGLOASGTEAETENFVHPTGSSRSCSSYELOOO
6	ECFIQFLSEP	TITFGTAIAAFYMPVTIMTILYW-	HISIASRSRVHKHRPEGPKEKKAKTIAFIKSPIMKQSVKKPPPGEA
7	QCFIQFLSNP	AVIFGTA IAAFYLPVVIMTVLYI-	RIYRETEKRTKOLADLOGSDSVYKAEKRKPAHRALFRSCLRCPRPT
8	ECQIQFLSEP	TITFGTA IAAFYIPVSIMTILYC-	KIIKEIEKKINDIADOQUSDSVIIVIEKUGVENINI
	RCYNDPKCCDFVINR NCYANETCCDFFINQ		RVFREAOKOVKKIDSCERRFLGGPARPPSPSPSPVPAPAPPGPPRP
9	RCYNDPKCCDFVINR TO THE PARTY OF	AYAIASSVVSFYVPLCIMAFVYL-	RVFQEAKROLOKIDKSEGREHVONLSOVEODGRTGHGLRRSSKFCL
10		AYA-ASSAVSFYVPLVIMVFVYS-	RVFVVATROLRLLRGELGRFPPEESPPAPSRSLAPAPVGTGAPPEG
11	RCHSNPRCCAFASNM———	PYVLLSSSVSFYLPLLVMLFVYA-	RVFVVATROLKDIRGEISIN FFEESFFILSION IN REPORTED REPORTED REPORT OF THE RE
12	ICQINEEP	GYVLFSALGSFYVPLTI1LVMYC-	RVYVVAKRESKILKSGLKIDASDSEQVILKIHOGAGYVEDSTS STK RVYIVAKRITKNLEAGVMKEMSNSKELTLRIHWSKNFHEDTLSSTK
13	ECVTEEP	FCALFCSLGSFYIPLAVILVMYC-	RIYRVAKRITKNIEAGVMALMSNSKEITIKTHWSKU IEDIDSTK RIYRVAKRRIRTISEKRAPVGPDGASPTTENGIGAAAGEARTGTAR
14	QCGLNDET-	WYILSSCIGSFFAPCLIMGLVYA-	RIYRVAKRETKILSEKKAPVGPDGASPITENGIGAAAGEAKTOTAK
15	OCKLNOEA	WYILASSIGSFFAPCLIMILVYL-	RIYLIAKRSNRRGPRAKGGPGOGESKOPRPDHGGALASAKLPALAS
16	RCEINDOK	WYVISSCIGSFFAPCLIMILVYV-	RIYQIAKRTRVPPSRRDPDAVAAPPGGTERRPNGLGPERSAGPGG
17	CCK I MUUK	WYVISSSIGSFFAPCLIMILVYV-	RIYQIAKRTRVPPSRRGPDACSAPPGGADRRPNAVGPERGAGTAG
18	DCCITCODI	GYVIYSSLGSFFIPLAIMTIVYI-	EIFVATRRIRERARANKINTIALKSTELEPMANSSPVAASNSGSK
19	NCDSSLSR	TYAISSSVISFYIPVAIMIVTYT-	RIYRIAQKQIRRIAALERAAVHAKNCQTTTGNKPVECSQPESSFKM
20	_CTOCCT NTD	TYAISSSLISFYIPVAIMIVTYT-	RIYRIAQVQIRRISSLERAAEHAQSCRSSAACAPDTSLRASIK—
21	ECI IANP-	AFVVYSSIVSFYVPFIVTLLVYI-	KIYIVLRRRKRVNTKRSSRAFRAHLRAPLKGNCTHPEDMKLCTVI
22	VCSISNP	DFVIYSSVVSFYLPFGVTVLVYA-	RIYVVLKQRRRKRILTRQNSQCNSVRPGFPQQSTSLPDPAHLELKR
23	VCRLEDR	DYVVYSSVCSFFLPCPLMLLLYW-	ATTRGLORWEVARRAKLHGRAPRRPSGPCPPSPTPPAPRLPODPCG
24	DCI IDEEO.	SYTIYSTCGAFYIPSVLLIILYG-	RIYRAARNRI LNPPSLYGKRFTTAHLI TGSAGSSLCSLNSSLHEGH
25	ACTISKDH	GYTIYSTIFAFYIPLLLMLVLYG-	RIFRAARFRI RKTVKKVEKTGADTRHGASPAPOPKKSVNGESGSRN
26	TCVLNDPN	FVLIGSFVA-FFIPTLIMVITYF-	LTIYVLRRQTIMLLRGHTEEELANMSLNFLNCCCKKNGGEEENAPN
27	SCLLADDN	FVLIGSFVA-FFIPLTIMVITYF-	LTIKSLOKEATLCVSDLSTRAKLASFSFLPQSSLSSEKLFQRSIHR
28	KCKVOVNE	VYGLVDGLVTFYLPLLIMCITYY-	RIFKVARDQAKRNHISSWKAATI
29	ACTENT SPWINDPKER	INVAVAMLTVRGI IRFI IGF SAPM	SIVAVSYGLIATKIHKQGL
30	LCGCDYSHDKRRER	AVAIVRLVLGFLWPLLTLTICYT-	FILLRTWSRRA- IIRCLSSSAVANRSKKSR
31	TCHDVLNETLLEGYYA	YYFSAFSAVFFFVPLIISTVCYVS	I IRCLSSSAVANRSKKSR
32	WCFLTLGAESGDVAFG	LLFSMLGGLSVGLSFLLNIVSVA-	
33	ACYEDMGNNYANWRM	LLRI LPQSFGFIVPLLIMLYCYGF	TLHHVYHGQEAAQQRUK———————————————————————————————————
34	RCF EHY EKGSKPV	LIIHICIVLGFFIVFLLILFCNL-	VIIHTLLRGPVKQQRNA
35	TCMLNATSKFMEFYQDV-KD		TLRTLFKAHM VIIHTLLRGPVKQQRNA— MICEMLNRNGSLRIALSEHL—
36	VCMINPFOKTAFMOFYKTAAKD	WWLFAFYFCLPLAITAIFYTL	MICEMINRRNGSIRIAISEHL MICEMIRKKSGM-QIAINDHL IARNIIQSAYNIPVEGNIHVKKQI IAKTLIRSAHNIPGEYNEHTKKQM
37	SCAPYPHSNELHPK	IHSMASFLVFYVIPLAIISVYYYF	IARNLIQSAYNLPVEGNIHVKKQI
38	ACIPYPOTDELHPK	THSVLIFLVYFLIPLVIISIYYYH	IAKTLIRSAHNLPGEYNEHTKKOM
39	YCRSFYPEHSIKEWLI	SMELVSVVLGFAVPFSIIAVFYFS	
40	VCTPIVDTATVK	VVIQVNTFMSFLFPMLVISILNT-	VIANKLTVMVHQAAEQGRVCTVGTHNGLEHSTFNMTIEPGRV
41	TCVIVYPSRSWEV	FTMMLLNLVGFLLPLSIITFCTVR	IMOVLENNEMKKFKEVO-
42	SCGYKI SRNYYS	PIYIMDEGVEYVVPMILATVLYGE	IARILFINPIPSDPKENSKMIKNDSIHONKNININA-
43	KCVVAWPEDSGGKTLL	LYHLVVIALIYFLPLAVMFVAYS-	VIGLTLWRRAVPGHQAHGANLRHL
44	VCMIEWPEHPNRTYEK	AYHICVTVLIYFLPLLVIGYAYT-	VVGITIWASEIPGDSSDRYHEQV
45	LCYV-WPEGPKQHF	TYHIIVIILVYCFPLLIMGVTYT-	IVGITLWGGEIPGDTCDKYHEQL
46	VCAFHYESQNSTLPV	GLGLTKNILGFLFPFLIILTSYT-	VIGITIMRAVPCHQARGANLRIL VVGITIMASEIPGDSSGNYHEQV IVGITIMGGEIPGDTCDKYHEQL LIWKTLKKAYEIQKNKP VVKIRKNTWAS-
47	DCRAVI	IFIAILSFLVFT-PLMLVSSTIL-	VVKIRKNTWAS-
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48	ICLPMDVETTLSO	-VYILTILILNVVAFLIICACYI-	
48 49	ICLPMOVETTLSQ	-VYILTILILNVVAFLIICACYI- -AYIVFVLTLNIVAFVIVCCCYV-	
49	TCT.PMOTETPI.AI	-AYIVFVLTLNIVAFVIVCCCYV-	
	ICLPMDVETTLSQ		KIYFAVRNPELMATN
49 50	ICLPMDIDSPLSQ	-AYIVFVLTLNIVAFVIVCCCYV-	KIYFAVRNPELMATN
49 50 51	ICLPMDTETPLAL	-AYİVFVLTLNIVAFVIVCCCYV- -LYVMSLLVLNVLAFVVICGCYT- SFVIYMFVVHFTIPMIIIFFCYG-	KIYFAVRNPELMATN
. 50 51 52	ICLPMDTETPLAL ICLPMDIDSPLSQ SCGIDYYTLKPEVNNE SCGPDVFSGSSYPGVQ	-AYİVFVLTLNIVAFVIVCCCYV- -LYVMSLLVLNVLAFVVICGCYT-	KIYFAVRNPELMATN
49 50 51 52 53	ICLPMOTETPLAL ICLPMOTESPLSQ SCGIDYYTLKPEVNNE SCGPDVFSGSSYPGVQ SCGPDVFSGSSYPGVQ	-AYIVFVLTLNIVAFVIVCCCYV- -LYVMSLLVLNVLAFVVICGCYT- SFVIYMFVVHFTIPMLIIFFCYG- SYMIVLMVTCCITPLSIIVLCYL-	KIYFAVRNPELMATN
49 50 51 52	ICLPMDTETPLAL ICLPMDIDSPLSQ SCGIDYYTLKPEVNNE SCGPDVFSGSSYPGVQ	-AYIVFVLTLNIVAFVIVCCCYV- -LYVMSLLVLNVLAFVVICGCYT- SFVIYMFVVHFTIPMIIIFFCYG- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLAIIMLCYL-	KIYFAVRNPELMATN- KIYITVRNPQYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAQQQESATTQ- QVWLAIRAVAKQQKESESTQ- QUWLAIRAVAKQQKESESTQ- QLLRALKAVAAQQQESATTQ-
50 51 52 53 54	ICLPMOTETPLAL ICLPMOTESPLSQ SCGIDYYTLKPEVNNE SCGPDVFSGSSYPGVQ SCGPDVFSGSSYPGVQ SCGPDWTVGTKYRSE	-AYIVFVLTLNIVAFVIVCCCYV- -LYVMSLLVLNVLAFVVICGCYT- SFVIYMFVVHFTIPMIIIFFCYG- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLAIIMLCYL-	KIYFAVRNPELMATN- KIYITVRNPQYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAQQQESATTQ- QVWLAIRAVAKQQKESESTQ- QVWLAIRAVAKQQKESESTQ- QLLRALKAVAAQQQESATTQ-
49 50 51 52 53 54	ICLPMDTETPLAL ICLPMDIDSPLSQ SCGIDYYTLKPEVNNE SCGPDVFSGSSYPGVQ SCGPDVFSGSSYPGVO SCGPDWTVGTKYRSE FCEPNQVIQLTCSDAFLND	-AYIVFVLTLNIVAFVIVCCCYV- -LYVMSLLVLNVLAFVVICGCYT- SFVIYMFVVHFTIPMIIIFFCYG- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCIIPLAIIMLCYL- SYTWFLFIFCFIVPLSLICFSYT-	KIYFAVRNPELMATN- KIYITVRNPQYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAQQQESATTQ- QVWLAIRAVAKQQKESESTQ- QUWLAIRAVAKQQKESESTQ- QLLRALKAVAAQQQESATTQ- KIVSSIC-
51 52 53 54 55 56	ICLPMDTETPLAL ICLPMDIDSPLSQ SCGIDYYTLKPEVNNE SCGPDVFSGSSYPGVQ SCGPDVFSGSSYPGVQ SCGPDWYTVGTKYRSE FCEPNQVIQLTCSDAFLND FCDGTPLLKLSCSDTHLNE	-AYIVFVLTLNIVAFVIVCCCYV- -LYVMSLLVLNVLAFVVICGCYT- SFVIYMFVVHFTIPMIIIFFCYG- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLAIIMLCYL- SYTWFLFIFCFIVPLSLICFSYT- LVIYFTLVLLATVPLAGIFYSYF- LMILTEGAVVMVTPFVCILISYI-	KIYFAVRNPELMATN— KIYITVRNPQYNPGD— HIYLTVRNPNIVSSS— QLVFTVKEAAAQQQESATTQ— QVWLAIRAVAKQQKESESTQ— QUWLAIRAVAKQQKESESTQ— QLLRALKAVAAQQQESATTQ— KIVSSIC— HITCAVL—
50 51 52 53 54 55 56 57	ICLPMOTETPLAL ICLPMOTESPLAQ SCGIDYYTLKPEVNNE SCGPDVFSGSSYPGVQ SCGPDVFSGSSYPGVQ SCGPDWYTVGTKYRSE FCEPNQVIQLTCSDAFLND FCDGTPLLKLSCSDTHLNE FCDISPWIVLSCTDTQVVE	-AYIVFVLTLNIVAFVIVCCCYVLYVMSLLVLNVLAFVVICGCYT- SFVIYMFVVHFTIPMIIIFFCYG- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCIIPLAIIMLCYL- SYMFLFIFCFIVPLSLICFSYT- LVIYFTLVLLATVPLAGIFYSYF- LMILTEGAVVMVTFFVCILISYI- LVSFGIAFCVILGSCGITLVSYA-	KIYFAVRNPELMATN- KIYITVRNPQYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAQQQESATTQ- QVWLAIRAVAKQQKESESTQ- QVWLAIRAVAKQQKESESTQ- QLLRALKAVAAQQQESATTQ- KIVSSIC- HITCAVL- YIITTII-
50 51 52 53 54 55 56 57 58	ICLPMOTETPLAL ICLPMOTETPLAL SCGIDYYTLKPEVNNE SCGPDVFSGSSYPGVQ SCGPDWFYGSSYPGVQ SCGPDWTVGTKYRSE FCEPNQVIQLTCSDAFLND FCDISPWIVLSCTDTQVVE FCELNQLSQLTCSDNFPSH	-AYIVFVLTLNIVAFVIVCCCYVLYVMSLLVLNVLAFVVICGCYT- SFVIYMFVVHFTIPMIIIFFCYG- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCIIPLAIIMLCYL- SYMFLFIFCFIVPLSLICFSYT- LVIYFTLVLLATVPLAGIFYSYF- LMILTEGAVVMVTPFVCILISYI- LVSFGIAFCVILGSCGITLVSYA- LIMNLVPVMLAAISFSGILYSYF-	KIYFAVRNPEIMATN- KIYITVRNPQYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAQQQESATTQ- QVWLAIRAVAKQQKESESTQ- QVWLAIRAVAKQQKESESTQ- QLLRALKAVAAQQQESATTQ- KIVSSIC- HITCAVL- YIITTII- KIVSSIH-
51. 52. 53. 54. 55. 56. 57. 58. 59.	ICLPMDTETPLAL ICLPMDIDSPLSQ SCGIDYYTLKPEVNNE SCGPDVFSGSSYPGVQ SCGPDVFSGSSYPGVO SCGPDWTVGTKYRSE FCEPNQVIQLTCSDAFLND FCDISPWLKLSCSDTHLNE FCDISPWLVLSCTDTQVVE FCELNQLSQLTCSDNFPSH FCDLFVLLKIACSDTYINE	-AYİVFVLTLNIVAFVIVCCCYV- -LYVMSLLVLAVLAFVVICGCYT- SFVIYMFVVHFTIPMLIIFFCYG- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLAIIMLCYL- SYMFLFIFCFIVPLSLICFSYT- LVIYFTLVLLATVPLAGIFYSYF- LMIFTMSTLLIIIPFFLIVMSYA-	KIYFAVRNPELMATN- KIYITVRNPQYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAQQQESATTQ- QVWLAIRAVAKQQKESESTQ- QVWLAIRAVAKQQKESESTQ- QLLRALKAVAAQQQESATTQ- KIVSSIC- HITCAVL- YIITTII KIVSSIH- RIISSIL-
51. 52. 53. 54. 55. 56. 57. 58. 59.	ICLPMOTETPLAL ICLPMOTESPLSQ SCGIDYYTLKPEVNNE SCGPDVFSGSSYPGVQ SCGPDVFSGSSYPGVQ SCGPDWTVGTKYRSE FCEPNQVIQLTCSDAFLND FCDISPMIVLSCTDTQVVE FCELNQLSQLTCSDNFPSH FCDLFVLLKLACSDTYINE FCDVSPLLNLSCTDMSTAE	-AYIVFVLTLNIVAFVIVCCCYV- -LYVMSLLVLNVLAFVVICGCYT- SFVIYMFVVHFTIPMIIIFFCYG- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLAIIMLCYL- SYMFLFIFCFIVPLSLICFSYT- LVIYFTLVLLATVPLAGIFYSYF- LMILTEGAVVMVTPFVCILISYI- LVSFGIAFCVILGSCGITLVSYA- LMIFIMSTILLIIIPFFLIVMSYA- LMIFIMSTILLIIIPFFLIVMSYA- LTDFVLAIFILLGPLSVTGASYM-	KIYFAVRNPELMATN- KIYITVRNPQYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAQQQESATTQ- QVWLAIRAVAKQQKESESTQ- QVWLAIRAVAKQQKESESTQ- QLLRALKAVAAQQQESATTQ- KIVSSIC- HITCAVL- YIITTII- KIVSSIH- RIISSIL- AITGAVM-
50 51 52 53 54 55 56 57 58 59 60 61	ICLPMOTETPLAL ICLPMOTETPLAL ICLPMOTESSO SCGIDYYTLKPEVNNE SCGPDVFSGSSYPGVQ SCGPDVFSGSSYPGVQ SCGPDWYTVGTKYRSE FCEPNQVIQLTCSDAFLND FCDGTPLLKLSCSDTHLNE FCDISPWIVLSCTDTQVVE FCELNQLSQLTCSDNFPSH FCDLFVLLKLACSDTYINE FCDUSPLLNLSCTDMSTAE FCDLFVLLKLACSDTYVNE	-AYIVFVLTLNIVAFVIVCCCYVLYVMSLLVLNVLAFVVICCCYV- SFVIYMFVVHFTIPMIIIFFCYG- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMFLFIFCFIVPLSLICFSYT- LVIYFTLVLLATVPLAGIFYSYF- LMILTEGAVVMVTPFVCILISYI- LVSFGIAFCVILGSCGITLVSYA- LIMILVPVMLAAISFSGILYSYF- LMIFIMSTLLIIIPFFLIVMSYA- LTDFVLAFFILLGPLSVTGASYM- LMIHIMGVIIIVIPFVLIVISYA-	KIYFAVRNPELMATN- KIYITVRNPOYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAQQQESATTQ- QVWLAIRAVAKQQKESESTQ- QVWLAIRAVAKQQKESESTQ- QLLRALKAVAAQQQESATTQ- KIVSSIC- HITCAVL- YIITTII KIVSSIH- RIISSIL- AITGAVM- KIISSIL-
50 51 52 53 54 55 56 57 58 59 60 61 62	ICLPMOTETPLAL ICLPMOTETPLAL ICLPMOTESSO SCGIDYYTLKPEVNNE SCGPDVFSGSSYPGVQ SCGPDVFSGSSYPGVQ SCGPDWYTVGTKYRSE FCEPNQVIQLTCSDAFLND FCDGTPLLKLSCSDTHLNE FCDLFVLLKLSCSDTYDE FCDLFVLLKLACSDTYNE FCDLFVLLKLACSDTYNE FCDLFVLLKLACSDTYNE FCDLFVLLKLACSDTYNE FCDMSTLLKVACSDTHDNE	-AYİVFVLTLNIVAFVIVCCCYV- -LYMSLLVLAVLAFVVICCCYT- SFVIYMFVVHFTIPMLIIFFCYG- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMFLFIFCFIVPLSLICFSYT- LVIYFTLVLLATVPLAGIFYSYF- LMILTEGAVVMVTPFVCILISYI- LVSFGIAFCVILGSCGITLVSYA- LIMILVPVMLAAISFSGILYSYA- LIMILVPVMLAAISFSGILYSYA- LMIFIMSTLLIIIPFFLIVMSYA- LMIFIMSTLLIIIPFVLIVISYA- LMIFILGGPIVVLPFLLIIVSYA- LAIFILGGPIVVLPFLLIIVSYA-	KIYFAVRNPELMATN- KIYITVRNPQYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAQQQESATTQ- QVWLAIRAVAKQQKESESTQ- QVWLAIRAVAKQQKESESTQ- QLLRALKAVAAQQQESATTQ- KIVSSIC- HITCAVL- YIITTII- KIVSSIH- RIISSIL- AITGAVM- KIISSIL- RIVSSIF-
49 50 51 52 53 54 55 56 57 58 59 60 61 62 63	ICLPMDTETPLAL ICLPMDIDSPLSQ SCGIDYYTLKPEVNNE SCGPDVFSGSSYPGVQ SCGPDVFSGSSYPGVQ SCGPDWTVGTKYRSE FCEPNQVIQLTCSDAFLND FCDISPLIKLSCSDTHLNE FCDISPULVLSCTDTQVVE FCELNQLSQLTCSDNFPSH FCDVSPLINLSCTDMSTAE FCDVSPLINLSCTDMSTAE FCDMSTLLKVACSDTWNE FCDMSTLLKVACSDTWNE FCDMSTLLKVACSDTWNE FCDMSTLLKVACSDTWNE FCDISALLKLSCSDIYVNE	-AYİVFVLTLNIVAFVIVCCCYV- -LYVMSLLVLAVLAFVVICGCYT- SFVIYMFVVHFTIPMLIIFFCYG- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMFLFIFCFIVPLSLICFSYT- LVIYFTLVLLATVPLAGIFYSYF- LMILTEGAVVMVTPFVCILISYI- LYMIVYMLAJISFSCILYSYF- LMIFIMSTLLIIIPFFLIVMSYA- LMIFIMSTLLIIIPFFLIVMSYA- LMIFIMGOPIVVLPFLLIVSYA- LAIFILGGPIVVLPFLLIVSYA- LMIYLIGGLIIIPFLLIVMSYV-	KIYFAVRNPELMATN- KIYITVRNPQYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAQQESATTQ- QVWLAIRAVAKQCKESESTQ- QVWLAIRAVAKQCKESESTQ- QLLRALKAVAAQQQESATTQ- KIVSSIC- HITCAVL- YIITTII KIVSSIH- RIISSIL- AITGAVM- KIISSIL- AITGAVM- KIISSIL- RIVSSIF- RIFFSIL-
50 51 52 53 54 55 56 57 58 59 60 61 62	ICLPMDTETPLAL ICLPMDIDSPLSQ SCGIDYYTLKPEVNNE SCGPDVFSGSSYPGVQ SCGPDVFSGSSYPGVQ SCGPDWTVGTKYRSE FCEPNQVIQLTCSDAFLND FCDISPLIKLSCSDTHLNE FCDISPULVLSCTDTQVVE FCELNQLSQLTCSDNFPSH FCDVSPLINLSCTDMSTAE FCDVSPLINLSCTDMSTAE FCDMSTLLKVACSDTWNE FCDMSTLLKVACSDTWNE FCDMSTLLKVACSDTWNE FCDISALLKLSCSDIYVNE	-AYİVFVLTLNIVAFVIVCCCYV- -LYMSLLVLAVLAFVVICCCYT- SFVIYMFVVHFTIPMLIIFFCYG- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMFLFIFCFIVPLSLICFSYT- LVIYFTLVLLATVPLAGIFYSYF- LMILTEGAVVMVTPFVCILISYI- LVSFGIAFCVILGSCGITLVSYA- LIMILVPVMLAAISFSGILYSYA- LIMILVPVMLAAISFSGILYSYA- LMIFIMSTLLIIIPFFLIVMSYA- LMIFIMSTLLIIIPFVLIVISYA- LMIFILGGPIVVLPFLLIIVSYA- LAIFILGGPIVVLPFLLIIVSYA-	KIYFAVRNPELMATN- KIYITVRNPQYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAQQQESATTQ- QVWLAIRAVAKQQKESESTQ- QVWLAIRAVAKQQKESESTQ- QLLRALKAVAAQQQESATTQ- KIVSSIC- HITCAVL- YIITTII- KIVSSIH- RIISSIL- AITGAVM- KIISSIL- RIVSSIF- RIFFSIL- RVVASIL-
49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64	ICLPMOTETPLAL ICLPMOTETPLAL ICLPMOTESSO SCGIDYYTLKPEVNNE——— SCGPDVFSGSSYPGVQ——— SCGPDVFSGSSYPGVQ——— SCGPDWTVGTKYRSE——— FCEPNQVIQLTCSDAFLND—— FCDGTPLLKLSCSDTHLNE—— FCDISPWIVLSCTDTQVVE—— FCELNQLSQLTCSDNFPSH—— FCDLFVLLKLACSDTYINE—— FCDLFVLLKLACSDTYVNE—— FCDMSTLLKVACSDTHDNE—— FCDISALLKLSCSDTYVNE—— FCDISPLLKLSCSDTYVNE—— FCDISPLLKLSCSDTYVNE—— FCDISPLLKLSCSDTYVNE——	-AYİVFVLTLNIVAFVIVCCCYVLYVMSLLVLNVLAFVVICGCYT- SFVIYMFVVHFTIPMLIIFFCYG- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMFLFIFFCFIVPLSLICFSYT- LVIYFTLVLLATVPLAGIFYSYF- LMILTEGAVVMVTPFVLILSYI- LWIFIMSTRLLIIIPFFLIVMSYA- LMIFIMSTLLIIIPFFLIVMSYA- LMIFILGGPIVVLPFFLLIVSYA- LMIFILGGLIIIIPFLLIVMSYV- LVIFVMGGLVIVIPFVLIIVSYA-	KIYFAVRNPELMATN- KIYITVRNPOYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAQQQESATTQ- QVWLAIRAVAKQQKESESTQ- QVWLAIRAVAKQQKESESTQ- QLLRALKAVAAQQQESATTQ- KIVSSIC- HITCAVL- YIITTII KIVSSIH- RIISSIL- AITGAVM- KIISSIL- RIVSSIF- RIFFSIL- RVVASIL-
49 50 51 52 53 54 55 56 57 58 59 60 62 63 64 65	ICLPMOTETPLAL ICLPMOTESPLSQ SCGIDYYTLKPEVNNE——————————————————————————————————	-AYİVFVLTLNIVAFVIVCCCYVLYYMSLLVLNVLAFVVICGCYT- SFVIYMFVVHFTIPMLIIFFCYG- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLAIIMLCYL- SYMFLFIFCFIVPLSLICFSYT- LVIYFTLVLLATVPLAGIFYSYF- LMITLTEGAVVMVTPFVLILSYI- LVSFGIAFCVILGSCGITLVSYA- LINNLVPVMLAAISFSGILYSYF- LMIFIMSTLLIIIPFFLIVMSYA- LMIFIMSTLLIIIPFFLIVMSYA- LMIFILGGPIVVLPFLLIIVSYA- LMIYILGGLIIIPFLLIVMSYV- LVIFVMGGLVIVIPFVLIVSYA- FWIGVTSVLLLFIVYAYMYILM-	KIYFAVRNPELMATN- KIYITVRNPOYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAOQQESATTQ- QVWLAIRAVAKQQKESESTQ- QVWLAIRAVAKQQKESESTQ- QULAIRAVAKQQKESESTQ- QLLRALKAVAAQQQESATTQ- KIVSSIC- HITCAVL- YIITTII- KIVSSIH- RIJSSIL- AITGAVM- KIISSIL- RIVSSIF- RIFFSIL- RVVASIL- KAHSHAVRMIQRGTQKSIIIHTSEDGKVQVTRPDQA-
49 50 51 52 53 54 55 56 57 58 59 60 62 63 64 65 66	ICLPMOTETPLAL ICLPMOTESPLSQ SCGIDYYTLKPEVNNE SCGPDVFSGSSYPGVQ SCGPDVFSGSSYPGVQ SCGPDVFSGSSYPGVQ SCGPDVFSGSSYPGVQ	-AYIVFVLTLNIVAFVIVCCCYVLYVMSLLVLNVLAFVVICGCYT- SFVIYMFVVHFTIPMLIIFFCYG- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLAIIMLCYL- SYMFLFIFCFIVPLSLICFSYT- LVIYPTLVLLATVPLAGIFYSYF- LMILTEGAVVMVTPFVLILSYI- LVSFGIAFCVILGSCGITLVSYA- LIMNLVPVMLAAISFSGILYSYF- LMIFIMSTILLIIPFFLIVMSYA- LAIFILGGPIVVLPFLLIVSYA- LAIFILGGPIVVLPFLLIVSYA- LMIYLGGLIIIPFFLLIVMSYV- LVIFVMGGLVIVIFFVLIVSYA- FWIGVTSVLLLFIVYAYMYILW YLDLATFILLYLLPLFIISVAYA-	KIYFAVRNPELMATN- KIYITVRNPQYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAQQQESATTQ- QVWLAIRAVAKQKESESTQ- QVWLAIRAVAKQKESESTQ- QULRALKAVAAQQQESATTQ- KIVSSIC- HITCAVL- YIITTII- KIVSSIH- RIISSIL- AITGAVM- KIISSIL- RIVSSIF- RIFFSIL- RVVASIL- KAHSHAVRMIQRGTQKSIIIHTSEDGKVQVTRPDQA- RVAKKLWICNTIGOVITEQYLALR-
49 50 51 52 53 54 55 56 57 58 90 61 62 63 64 66 67	ICLPMOTETPLAL ICLPMOTETPLAL ICLPMOTESSO SCGIDYYTLKPEVNNE——— SCGPDVFSGSSYPGVQ——— SCGPDVFSGSSYPGVQ——— SCGPDWYTVGTKYRSE——— FCEPNQVIQLTCSDAFLND—— FCDGTPLLKLSCSDTHLNE—— FCDLFVLLKLACSDTYINE—— FCDLFVLLKLACSDTYNNE—— FCDMSTLLKVACSDTYNNE—— FCDMSTLLKVACSDTYNNE—— FCDISALLKLSCSDTYNNE—— FCDISPLLKLSCSDTYNNE—— VCCDIFPLIDGTYIM——— UCCDIFPLIDGTYIM——— LUDPFPEPADLFWK————	-AYIVFVLTLNIVAFVIVCCCYVLYVMSLLVLNVLAFVVICCCYVLYVMSLLVLNVLAFVVICCCYT- SFVIYMFVVHFTIPMIIIFFCYG- SYMIVLMVTCCIIPLSIIVLCYL- SYMIVLMVTCCIIPLSIIVLCYL- SYMIVLMVTCCIIPLAIIMLCYL- SYMFLFIFCFIVPLSLICFSYT- LVIYFTLVLLATVPLAGIFYSYF- LMILTEGAVVMVTPFVCILISYI- LVISFGIAFCVILGSCGITLVSYA- LMIHMEVILILIPFFLIVMSYA- LMIFILGCPIVVLPFVLIVISYA- LMIFILGCPIVVLPFLLIVMSYV- LVIFVMGGLVIVIPFVLIVISYA- FWIGVTSVLLLFIVYAYMYILM- SYTILLIVLOYFOPLFFIFICYF-	KIYFAVRNPELMATN- KIYITVRNPOYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAQQESATTQ- QVWLAIRAVAKQQKESESTQ- QVWLAIRAVAKQQKESESTQ- QLLRALKAVAAQQQESATTQ- KIVSSIC- HITCAVL- YIITTII- KIVSSIH- RIISSIL- AITGAVM- KIISSIL- RIVSSIF- RIFFSIL- RVVASIL- KAHSHAVRMIQRCTQKSIIIHTSEDGKVQVTRPDQA- RVAKKLWICNTIGDVTTEQYLALR- KIYIRLKRRNNMMDKIRDSKYRSS-
49 50 51 52 53 54 55 56 57 58 59 60 62 63 64 65 66 66 68	ICLPMOTETPLAL ICLPMOTETPLAL ICLPMOTESSO SCGIDYYTLKPEVNNE——— SCGPDVFSGSSYPGVQ——— SCGPDVFSGSSYPGVQ——— SCGPDWYTVGTKYRSE——— FCEPNQVIQLTCSDAFLND—— FCDGTPLLKLSCSDTHLNE—— FCDLFVLLKLACSDTYINE—— FCDLFVLLKLACSDTYNNE—— FCDMSTLLKVACSDTYNNE—— FCDMSTLLKVACSDTYNNE—— FCDISALLKLSCSDTYNNE—— FCDISPLLKLSCSDTYNNE—— VCCDIFPLIDGTYIM——— UCCDIFPLIDGTYIM——— LUDPFPEPADLFWK————	-AYIVFVLTLNIVAFVIVCCCYVLYVMSLLVLNVLAFVVICCCYVLYVMSLLVLNVLAFVVICCCYT- SFVIYMFVVHFTIPMIIIFFCYG- SYMIVLMVTCCIIPLSIIVLCYL- SYMIVLMVTCCIIPLSIIVLCYL- SYMIVLMVTCCIIPLAIIMLCYL- SYMFLFIFCFIVPLSLICFSYT- LVIYFTLVLLATVPLAGIFYSYF- LMILTEGAVVMVTPFVCILISYI- LVISFGIAFCVILGSCGITLVSYA- LMIHMEVILILIPFFLIVMSYA- LMIFILGCPIVVLPFVLIVISYA- LMIFILGCPIVVLPFLLIVMSYV- LVIFVMGGLVIVIPFVLIVISYA- FWIGVTSVLLLFIVYAYMYILM- SYTILLIVLOYFOPLFFIFICYF-	KIYFAVRNPELMATN- KIYITVRNPOYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAQQQESATTQ- QVWLAIRAVAKQQKESESTQ- QVWLAIRAVAKQQKESESTQ- QULRALKAVAAQQQESATTQ- KIVSSIC- HITCAVL- YIITTII KIVSSIH- RIISSIL- AITGAVM- KIISSIL- RIVSSIF- RIFFSIL- RVVASIL- KAHSHAVRMIQRGTQKSIIIHTSEDGKVQVTRPDQA- KIYIRLKRRNNMMCKIRDSKYRSS- RIYSLVRTRSRRLTTRKNISKASRS-
49 50 51 52 53 54 55 56 57 58 59 60 62 63 64 65 66 66 66 66	ICLPMOTETPLAL ICLPMOTETPLAL ICLPMOTESSO SCGIDYYTLKPEVNNE——————————————————————————————————	-AYİVFVLTLNIVAFVIVCCCYVLYYMSLLVLAVLAFVVICCCYVLYYMSLLVLAVLAFVVICCCYT- SFVIYMFVVHFTIPMLIIFFCYG- SYMIVLMYTCCITPLSIIVLCYL- SYMIVLMYTCCITPLSIIVLCYL- SYMIVLMYTCCITPLSIIVLCYL- SYMFLFIFCFIVPLSLICFSYT- LVIYFTLVLLATVPLAGIFYSYF- LMILTEGAVVMYTPFVLLILSYI- LIMHLVPVMLAAISFSGILYSYF- LMIFIMSTLLIIIPFFLIVMSYA- LTDFVLATFILLGPLSVTGASYM- LMIHIMGVIIVIPFVLIVISYA- LMIYILGGLIIIPFLLIVMSYV- LVIFVMGGLVIVIPFVLIVSYA- FWIGVTSVLLLFIVYAYMYILM- YIDLATFILLYLDYFIISVAYA- SYTTLLLVLOYFOPLEFIFICYFYMFFSFITWILIPLWMXIYLIV	KIYFAVRNPEIMATN- KIYITVRNPOYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAOQQESATTQ- QVWLAIRAVAKQKESESTQ- QVWLAIRAVAKQCKESESTQ- QULRALKAVAAQQQESATTQ- KIVSSIC- HITCAVL- YIITTII- KIVSSIH- RIJSSIL- RIVSSIL- RIVSSIL- RIVSSIF- RIFFSIL- RVVASIL- KAHSHAVRMIQRCTQKSIIIHTSEDGKVQVTRPDQA- RVAKKLWICHTIGDVTTEQYLALR- KIYIRLKRNNMMDKIRDSKYRSS- RIYSLVRTRSRRLTFRKNISKASRS- IFYIIRNKLSQNLTGFRETRAFYG-
49 50 51 52 53 54 55 56 57 58 60 61 62 63 64 65 67 68 69 70	ICLPMOTETPLAL ICLPMOTESPLSQ SCGIDYYTLKPEVNNE	-AYIVFVLTLNIVAFVIVCCCYVLYVMSLLVLNVLAFVVICCCYVLYVMSLLVLNVLAFVVICCCYT- SFVIYMFVVHFTIPMLIIFFCYG- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLAIIMLCYL- SYMFLFIFCFIVPLSLICFSYT- LVIYFTLVLLATVPLAGIFYSYF- LMILTEGAVVMVTPFVLILSYI- LVISFGIAFCVILGSCGITLVSYA- LIMNLVPVMLAAISFSGILYSYF- LMIFIMSTILLIIPFFLIVMSYA- LAIFILGGPIVVLPFLLIVSYA- LAIFILGGPLVVLPFLLIVSYA- LMIYLGGLIIITPFLLIVMSYV- LVIFVMGGLVIVIPFVLIVSYA- FWIGVTSVLLLFIVYAYMYILM- YLDLAFFILLYLLFIFISVAYA- SYTTLLLVLQYFGPLCFIFCYFYHLFCTTVFTLLLLSIVILYCYMFFSFITWILIPLVVMCIIYLD GFVLYTFMGFLLPVGAICLCTV-	KIYFAVRNPELMATN- KIYITVRNPOYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAQQQESATTQ- QVWLAIRAVAKQQKESESTQ- QVWLAIRAVAKQQKESESTQ- QLLRALKAVAAQQQESATTQ- KIVSSIC- HITCAVL- YIITTII- KIVSSIH- RIISSIL- AITGAVM- KIISSIL- RIVSSIF- RIFFSIL- RVVASIL- KAHSHAVRMIQRGTQKSIIIHTSEDGKVQVTRPDQA- RVAKKLWICKTIGDVTTEQYLALR- KIYIRLKRRNNMMDKIRGSKYRSS- RIYSLVRTRSRLTFRKNISKASRS- IFYIIRNKLSQNLTGFRETRAFYG- LIIAKMRMVALKAGWQQRKR-
49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 66 67 71	ICLPMOTETPLAL ICLPMOTESPLSQ SCGIDYYTLKPEVNNE——— SCGPDVFSGSSYPGVQ——— SCGPDVFSGSSYPGVQ——— SCGPDVFSGSSYPGVQ——— SCGPDWYTVGTKYRSE——— FCEPNQVIQLTCSDAFLND—— FCDISPHIVLSCIDTOVVE—— FCDLSQLTCSDNFPSH—— FCDLFVLLKLACSDTYLNE—— FCDLFVLLKLACSDTYVNE—— FCDMSTLLKVACSDTYVNE—— FCDISALLKLSCSDTYVNE—— FCDISALLKLSCSDTYVNE—— FCDISPLLKLSCSDTYVNE—— VCCDIFPLINGTYLM—— UCLIPFPEPADLFWK——— VCFDKFPSDSHRL——— SCSTVLPLYHKH——— SCHFRSVVGLD——— ACNIMMO———————————————————————————————————	-AYIVFVLTLNIVAFVIVCCCYVLYVMSLLVLNVLAFVVICCCYVLYVMSLLVLNVLAFVVICCCYT- SFVIYMFVVHFTIPMIIIFFCYG- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- LVIYFTLVLLATVPLAGIFYSYF- LMITEGAVVMVTPFVLILISYI- LWISTGIAFCVILGSCGITLWSYA- LIMILWSYMLAAISFSGILYSYA- LMIFILGGPIVVLPFLLIVMSYA- LMIFILGGLIIIPFFLLIVMSYA- LWIFLGGLIVLFFVLIVSYA- LWIFLSTSVLLLFIVYAYMYILW- YLDLATFILLVLYFFFISVAYA- SYTTLLLVLQYFCPLCFFFTCYFYILFCTTVFTLLLLSIVILYCYMFFSFITWILTPLWMCITYLD GFVLYTFIMGFLLPVGCILCYV- ISLGILLFFLFC-PHWIPCLAL-	KIYFAVRNPELMATN- KIYITVRNPOYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAQQQESATTQ- QVWLAIRAVAKQQKESESTQ- QVWLAIRAVAKQQKESESTQ- QLLRALKAVAAQQQESATTQ- KIVSSIC- HITCAVL- YIITTII- KIVSSIH- RIISSIL- AITGAVM- KIISSIL- RIVSSIF- RIFFSIL- RVVASIL- KAHSHAVRMIQRGTQKSIIIHTSEDGKVQVTRPDQA- RVAKKLWICKTIGDVTTEQYLALR- KIYIRLKRRNNMMDKIRGSKYRSS- RIYSLVRTRSRLTFRKNISKASRS- IFYIIRNKLSQNLTGFRETRAFYG- LIIAKMRMVALKAGWQQRKR-
49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 70 71 72	ICLPMDTETPLAL ICLPMDIDSPLSQ SCGIDYYTLKPEVNNE——————————————————————————————————	-AYİVFVLTLNIVAFVIVCCCYVLYVMSLLVLNVLAFVVICCCYVLYVMSLLVLNVLAFVVICCCYT- SFVIYMFVVHFTIPMLIIFFCYG- SYMIVLMVTCCIIPLSI IVLCYL- SYMIVLMVTCCIIPLSI IVLCYL- SYMIVLMVTCCIIPLAI IMLCYL- SYMFLFIFCFIVPLSLICFSYT- LVIYFTLVLLATVPLAGIFYSYF- LMILTEGAVVMVTPFVLILISYI- LVISFGIAFCVILGSCGITLVSYA- LIMILVPVMLAAISFSGILYSYA- LMIFINGSTLLIIIPFFLIVMSYA- LMIFILGOPIVVLPFVLIVISYA- LMIFILGOPIVVLPFVLIVISYA- LMIFILGGLIVIPFVLIVISYA- FWIGVTSVLLLFIVYAYMY'LM- YLDLATFILLYLPLFIISVAYA- SYTTLLLVLQYFOPLGFIFICYFYILFCTTVFTLLLLSIVILYCYMFSFITWILIPLWWCIIYLD GFVLYTFILGFLFVCGLCYV- ISLGILLFFLFC-PLWVLPCLAL- SWYLLITMWGAAPVIMMIWFYA-	KIYFAVRNPELMATN- KIYITVRNPOYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAQQQESATTQ- QVWLAIRAVAKQQKESESTQ- QVWLAIRAVAKQQKESESTQ- QLLRALKAVAAQQQESATTQ- KIVSSIC- HITCAVL- YIITTII- KIVSSIH- RIISSIL- AITGAVM- KIISSIL- RIVSSIF- RIFFSIL- RVVASIL- KAHSHAVRMIQRGTQKSIIIHTSEDGKVQVTRPDQA- RVAKKLWICKTIGDVTTEQYLALR- KIYIRLKRRNNMMDKIRGSKYRSS- RIYSLVRTRSRLTFRKNISKASRS- IFYIIRNKLSQNLTGFRETRAFYG- LIIAKMRMVALKAGWQQRKR-
49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 66 67 71	ICLPMOTETPLAL ICLPMOTETPLAL ICLPMOTESSO SCGIDYYTLKPEVNNE— SCGPDVFSGSSYPGVQ— SCGPDVFSGSSYPGVQ— SCGPDWFYVGTKYRSE— FCEPNQVIQLTCSDAFLND— FCDISPLIKLSCSDTHLNE— FCDISPLIVLSCTDTQVVE— FCDLFVLLKIACSDTYINE— FCDLFVLLKIACSDTYINE— FCDLFVLLKIACSDTYVNE— FCDLSALLKLSCSDTYVNE— FCDISPLLKLSCSDTHONE— VCCDIFPLIDGTYLM— VCCDIFPLIDGTYLM— VCFDKFPSDSHRL— SCSTVLPLYHKH— SCHFRSVVGLD— ACNMIMPEPAQRVLV— ACLMMO— TCVLYFVAEEVHTVLL— TCVLYFVAEEVHTVLL— TCVLYFVAEEVHTVLL— TCVLYFVAEEVHTVLL— TCVLYFVAEEVHTVLL— SCGUMPANETSGWFPV— TCVLYFVAEEVHTVLL— TCVLYFVAEVHTVLL— TCVLYF	-AYIVFVLTLNIVAFVIVCCCYVLYVMSLLVLNVLAFVVICCCYVLYVMSLLVLNVLAFVVICCCYT- SFVIYMFVVHFTIPMIIIFFCYG- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- SYMIVLMVTCCITPLSIIVLCYL- LVIYFTLVLLATVPLAGIFYSYF- LMITEGAVVMVTPFVLILISYI- LWISTGIAFCVILGSCGITLWSYA- LIMILWSYMLAAISFSGILYSYA- LMIFILGGPIVVLPFLLIVMSYA- LMIFILGGLIIIPFFLLIVMSYA- LWIFLGGLIVLFFVLIVSYA- LWIFLSTSVLLLFIVYAYMYILW- YLDLATFILLVLYFFFISVAYA- SYTTLLLVLQYFCPLCFFFTCYFYILFCTTVFTLLLLSIVILYCYMFFSFITWILTPLWMCITYLD GFVLYTFIMGFLLPVGCILCYV- ISLGILLFFLFC-PHWIPCLAL-	KIYFAVRNPELMATN- KIYITVRNPQYNPGD- HIYLTVRNPNIVSSS- QLVFTVKEAAAQQQESATTQ- QVWLAIRAVAKQKESESTQ- QVWLAIRAVAKQKESESTQ- QVWLAIRAVAKQQESATTQ- KIVSSIC- HITCAVL- YIITIII- KIVSSIH- RIISSIL- AITGAVM- KIISSIL- RIVSSIF- RIFFSIL- RVASIL- KAHSHAVRMIQRGTQKSIIIHTSEDGKVQVTRPDQA- RVAKKLWICHTIGDVITEGYLALR- KIYIRLKRRNNMPKIRDSKYRSS- RIYSLVATSRRLTFRKNISKASRS- IIYIIRNKLSQNLTGFRETRAFYG- LIIAKMRWALKAGWQQRKR- LHVECRARRRQ- FFYSTVQRTSQ- RWYRFIINTYG-

			_
1	KEKHLTYQFK	LINYIIVFLVCWVFAVVNRIVNGL	nmfppalnilhtyl
2	KEVHAAKS	LALIUGLFALCWLPLHIINCFTFF LALIUFLFALSWLPLHIINCITLF	CPECSHAPLW
,		EADILE DE ADSHDE LA LOCCI LE	CF3CKKF31
4	(83) -KGOKPRGKEQLAKRKTFSLVKEKKAART		CKDCVPET
5	(110) -K-IVKMTK-QPAKKKP-PPSREKKVTRT		CAPCIPNT
6 7	(166) - KRFALKTRSQITKRKRMSLVKEKKAAQT		CDSCIPKT
8	(113) – K-Fasiarnovrkkrom-aarerkvtrt (155) – Kginpnpshomtkrkmsivkerkaaot	IFAILLAFILTWTPYNVMVLVNTF LSAILLAFIITWTPYNIMVLVSTF	CDKCVPVT
-	(200)		1
9	-AAAAATAPLANGRAGKRRPSRLVALREQKALKT		HRELVPDR
10	KehkalktVPACGRRPARLIPIREHRALCT	LGIIMGTFTLCWLPFFIVNIVHVI	QDNLIRKE
11 12	VPACGRRPARLIPIREHRALCT	IGLIMGTFTLCWLPFFLANVLRAL IGIVVGCFVLCWLPFFLVMPIGSF	GGPSLVPGPFPDFRPSET
13	AKGHNPRSSIAVKLFKFSREKKAAKT	LGIVVGMFILCWLPFFIALPLGSL	FSTLKPPDA
14	-(77)-FLSRRRRARSSVCRRKVAQAREKRFTFV	LAVVMGVFVLCWFPFFFIYSLYGI	CREACOVPGP
15	- (106) - GRGVGAIGGQWWRRRAHVTREKRFTFV	LAVVIGVFVLCWFPFFFSYSLGAI	CPKHCKVPHG
16	- (84) - GRGRSASGLPRRRAGAGGONREKRFTFV	LAVVIGVEVVCWEPFFFTYTLTAV	GCSVPRT
17 18	- (84) -GQGEERAGGAKASRWRGRQNREKRFTFV- (167) -KKTSGVNQFIEEKQKISLSKERRAART	LAVVIGVFVVCWFPFFFTYTLIAV LGIIMGVFVICWLPFFLMYVILPF	
19	SFKRETKVLKT	LSVIMGVEVCCWLPFFILNCILPF	CGSGETQPFCIDSN
20	SFKRETKVLKT KETKVLKT	LSVIMGVFVCCWLPFFILNCMVPF	CSGHPEGPPAGFPCVSET
21	- (91) - PNGKTRTSLKTMSREKLSQQKEKKATQM	LAIVLGVFIICWLPFFITHILNIH	CDCNIPPV
22	- (47) - SNGRLSTSLKLPLQPRGVPLREKKATQM	VAIVLGAFIVCWLPFFLTHVLNTH	COTCHVSPE
23 24	- (29) -ALPPQTPPQTRRRRAKITGRERKAMRV- (10) -NHVKIKLADSALERKRISAARERKATKI	LPVVVGAFILCWTPFFVVHITQAL LGIILGAFIICWLPFFVVSLVLPI	CPACSVPPR
25	- (57) -ASFERKNERNAEAKRKMALARERKTVKT	LGI IMGITILCWLPFFIVALVLPF	CESSCHIPTL
26	-NPNPDQKPRRKKKEKRPRGTMQAINNEKKASKV	LGIVFFVFLIMWCPFFITNILSVL	CGKACNQLMEK
27	EPGSYAGRKTMQSISNEQKACKV	LGIVFFLFVVMCPFFITNIMAVI	CKESCNENVIGA
28	REHKATVT	LAAVMGAFIICWFPYFTAFVYRGL	RGDDAINEV
29	TKSSRDT.BU	LSFVAAAFFLCWSPYQVVA LIATV	-RIRELLOGMYKEIGI
30	——————————————————————————————————————	VVAVVASFFIFWLPYQVTGIMMSF	LEPSSPTFLLLNK
31	TNRCFNSTV	ALFLSAAVFCIFIICFGPTNVLLI	AHYSFLSHTSTTEAAYF
32 33		LLGIMVVASVCVLPLLVFIAQTVL	-RNPPAMSPAGQLSRITEKE
33 34		IFAVVLIFLLCWLPYNLVLLADTL VCTVLAVFVICFVPHHMVQLPWTL	MRTQVIQETCERRNHIDR
35	KORREVAKT	VFCLVVIFALCWFPLHLSRILKKT	VYDEMDTNRCELLSFLIL.
36		VFCLVLVFALCWLPLHLSRILKLT	LYDQSNPQRCELLSFLLV
37	ESRKRLAKT	VLVFVGLFAFCWLPNHVIYLYRSY	HYSEVDTSMIJIEV
38 39	ETRKRLAKI	VLVFVGCFVFCWFPNHILYLYRSF	NYKEIDPSLGHMI
40		IFSYVVVFLVCWLPYHVAVLLDIF LRAVVIAFVVCWLPYHVRRLMFCY	SILHYIPFTCRLEHALFT ISDEOWTTFLFDFYHY
41	TEKKATVL	VLAVIGLEVICWEPFOISTFLDTL	-LRLGVLSGCWNERAVDI
42	SSRKQVTKM	LAVVVILFALLMMPYRTLVVVNSF	LSSPFQENWK
43 44	QAKKFVKT	MVLVVVTFAICWLPYHLYFILGSF	QEDIYCHKFIQQ
45		MIVVVCTFAICWLPFHVFFLLPYI MIVVVTFAICWLPYHVYFILTAI	YQQLNRWKY1QQ
46	RKDDIFKI	ILAIVLFFFFSWVPHNIFTFMDVL	-IOLGLIROCKIEDIVOT
47	IKSSRPLRV TRSTKTLKV TRNCFNSTV DSEVEMMO GCKHRAMRV EVRRALMM KORREVAKT SERKLAKT ESRKLAKT OALRHGVLV TEKKATVL SAKRKVVKM KAKRKVVKM KAKRKVVKM RAKRKVVKM HSSKLYIV	INVTITIFLIFAMPMRLLYLLYYE	-IQLGLIRDCKIEDIVDTYWSTFGN
40			
48 49	KDTKIAKKM	AILIFTDFT-CMAPISFFAISAAF AVLIFTDFI-CMAPISFYALSAIL	KVPLITVTNSK
50	SDTRIAKRM	AMLIFTOFL-CMAPISFFAISASL	NKPLITVSNSK
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51	KAEKEVTRM	VIIMVIAFLICWVPYASVAFYIFT	HOGSNFGPI
52 53		VVVMVLAFCFCWGPYAFFACFAAA	NPGYPFHPL
54		WWW.GSPCVCYUPYBBFBWWW.ii	NPGYAFHPLNRNHGLDLR
55	——————————————————————————————————————	AFSTCASHLSVVSLFYCTGLGVYL	SSAANNSSQASA
56	RVSSPRGGWK	SFSTCGSHLAVVCLFYGTVIAVYF	NPSSSHLÄGROM
57 58	KIPSARGRIR	AFSTCSSHLTVVLIWYGSTIFLHV	RTSVESSLDLTK
59	KVPSTOGICK	Afstcashlsivslfystglgvyv Vfstcgshlsvvslfygti iglyl	SSAVVQSSHSAA CPAGNNSTVKEM
50	RIPSAAGRHK	AFSTCASHLTVVI IFYAASIFIYA	RPKALSAFTDNK
ภ		VFSTCGSHLSVVSLFYGTI IGLYL	CPSGDNFSLKGS
52	KVPSSQSIHK	AFSTCGSHLSVVSLFYGTVIGLYL	CPSANNSEVKETCPSGNNSTVKEI
53 :4		VFSTCGSHLSVVTLFYGTIFGIYL	CPSGNNSTVKEI
54			CPSANNSTVKET
55	RMDI RLAKT	LVLILVVLIICWGPLLAIMVYDVF	GKMNKLIKT
56		LVLVVVLFALCWFPLNCYVLLLS-	SKAIHTNNA
77	ETKRINV-M		NHQIIATCNHNL
8 :e	SENVALLKT	VIIVLSVFIACWAPLFILLLIDVG	CKVKTCDILFR
9	REFKTAKS	LFLVLFLFALCWLPLSIINFVSYF	NVKI PET
1	RSAKI NIKV	VMMVMVFVICWMPFYVVQLVNVF VIAIVSVFLV-SSIYLGIDWFLFW	AEQDDAT
2	VORCETTE	VSVLLISFVALQTPYVSLMIFNSY	ATTAWPMOCFHITIRRT
3		LLVVVVSFASFWFPFNLALFLESI	RLLAGVYNDTLONVIIF
14	RHKGRIVRV	LIAVVLVFIIFWLPYHLTLFVDTI	KLLKWISSSCEFERSLKR

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GAKILTVFTFFGYFTDVQKKLEKNKNNNNPSPYSSSRGTSGKTMGGHPTGDDVQCSSDMEQCSLERHPNMV-(63)
     SVSHGFWASVTF IYNNPIM-WRYF
                               IREFROTFRKIIRSHVLRRREPFKAGGTSARALAAHGSDGEQISLRLNGHPPGVWANGSAPHPERRPNGYT- (50)
     LMYLTIVLSHTNSVVNPFI-YAYR
                               I OKFRVTF LK I WNDHFRCOPTPPVDEDPPEEAPHD
     LMY IAIFLTHGNSAMNPIV-YAFR
                                NKAFROTFRLLLLCRWOKRRWRK1PKRPGSVHRTPSRQC
     LWELGYWLCYVNSTINPMC-YALC
     WTIGYWLCYINSTINPAC-YALC
                               NATEKKTEKHLIMCHYKNIGATR
                                nktfrttfktlllcocdkrkrrkooyoorosvifhkrvpeqal
     YWNLGYWLCYINSTVNPVC-YALC
     VWSIGYWLCYVNSTINPAC-YALC
                                NATEKKTERHLLLCORYNIGTAR
                                nrtfrktfkmlllcrwkkkkveeklywognsklp
     LWHLGYWLCYINSTVNPIC-YALC
                                PDFRKAFQGLLCCARRAARRHATHGDRPRASGCLARPGPPSPGAASDDDDDDVVGATPPARLLEPWAGCN- (25)
     Leveenwlgyansafnpii-ycrs
9
                                PDFRIAFOELLCLRRSSLKAYGNGYSSNGNTGEQSGYHVEQEKENKLLCEDLPGTEDFVGHQGTVPSDNID-(13)
     VYILLNWIGYVNSGFNPLI-YCRS
10
                                PDFRSAFRRILCRCGRRLPPEPCAAARPALFPSGVPAAESSPAQPRICQRLDG
11
     AFLALNWLGYANSAFNPLI-YCRS
                                SQEFKKAFQNVLRIQCLRRKQSSKHTLGYTLHAPSHVLEGQHKDLVRIPVGSAETFYKISKTDGVCEWKIF- (66)
     VFKIAFWLGYLNSCINPII-YPCS
12
                                SKEFKRAFMRI LCCQCRGGRRRRRRRLGACAYTYRPWTRCGSLERSQSRKDSLDDSGSCMSGQKRTLPSA- (93)
13
14
     vekvvewlgyfnscinpii-ypcs
                                NODFRESFKHILFRRRRRGFRO
     LFKFFFWIGYCNSSLNPVI-YTVF
                                NODFRRAFRRILCRPWTQTAW
     LFQFFFWIGYCNSSLNPVI-YTIF
15
                                NHDFRRAFKKILCRGDRKRIV
     LFKFFFWFGYCNSSLNPVI-YTIF
16
                                NHDFRRAFKKILCRGDRKRIV
     LFNFFFWFGYCNSSLNPVI-YTIF
17
     FKNFITWLGYINSGLNPVI-YTIF
                                NLDYRRAFKRLLGLN
18
                                NADFRKAFSTLLGCYRLCPATNNAIETVSINNNGAAMFSSHHEPRGSISKECNLVYLIPHAVGSSEDLKKE- (42)
19
20
21
     TFDVFVWFGWANSSLNPII-YA-F
                                nadfokvfaqllgcshfcsrtpvetvnisnelisynodivfhkeiaaayihmpnavtpgnrevdndeeeg- (45)
     TFDVFVWFGWANSSLNPVI-YA-F
     LYSAFTWLGYVNSAVNPI I -YTTF
                                NIEFRKAFLKILHC
     LYSATTWLGYVNSALNPVI-YTTF
                                NTEFRKAFIKTLSC
22
                                NAFFRNVFRKALRACC
     LVSAVTWLGYVNSALNPVI-YTVF
                                NEEFROAFOKIVPFRKAS
24
     LFDFFTWLGYLNSLINPII-YTVF
                                NKDFQNAFKKI I KCNFCRQ
     IGAI INWLCVINSLLNPVI -YAYF
25
                                nkiyrrafskylrcdykpdkkppvrqiprvaatalsgrelavniyrhtnervarkandpepgienqvenle- (16)
     LLNVFVWIGYVCSGINPVI-YTLF
26
                                nktyrsafsrylocoykenrkplolilvntipalaykssolovgokknsoedaeotvddcsmytlgkoose- (17)
     LINVEVWIGYLSSAVNPLV-YTLF
27
                                NRDFRTGYQQLFCCRLANRNSHKTSLRSNASQLSRTQSREPRQQEEKPLKLQVWSGTEVTAPQGATDR
     LEAIVLWLGYANSALNPIL-YAAL
28
                                GODFRERLIHALPASLERALTEDSTOTSDTATNSTLPSAEVALQAK
     AVDVTSALAFFNSCLNPML-YVFM
29
                                GOGOFOGRIRKSLPSLIRNVLTEESVVRESKSFTRSTVDTMAOKTOAV
30
     LDSLCVSFAYINCCINPII-YVVA
                                SSECORYVYSILCCKESSDPSSYNSSCOLMASKADTCSSNLNNSIYKKLLT
     AYLLCVCVSSISSCIDPLI-YYYA
31
                                RRAVLRRLOPRLSTRPRSLSLOPQLTQRSGLO
32
     -Lliylrvatwnqildpwv-yilf
                                GOKFRHGLLKILAIHGLISKDSLPKDSRPSFVGSSSGHTSTTL
     ALDATEILGILHSCLNPLI-YAFI
                                TKKFRKHLSEKLNIMRS SQKCSRVTTDTGTEMAIPINHTPVNPIKN
     AHQVTLCLLSTNCVLDPVI-YCFL
34
                                KKFKNCFQSCLCCCCYQSKSLMTSVPMQGTSIQWKNHEQNNHNTERSSHKDSIN
     MDYIGINLATMNSCINPIALYFVS
35
     LDYIGINMASLNSCINPIALYLVS
                                KRFKNCFKSCLCCWCQTFEEKQSLEEKQSCLKFKANDHGYDNFRSSNKYSSS
36
37
38
                                KSERQFNTQLLCCQPGLMNRSHSTGRSTTCMTSFKSTNPSATFSL1NRN1CHEGYV
     TSICARLIAPTNSCVNPFALYLLS
                                ESFRKHFSNQLCCGQKSYPERSTSYLLSSSAVRMTSLKSNAKNVVTNSVLLNGHSTKQEIAL
     VTLVARVLSFSNSCVNPFALYLLS
                                nrnyryelmkaf if ky saktgltklidas rvsetey saleqnak
     ALHVTQCLSLVHCCVNPVL-YSFI
39
                                SANFROVFLSTLACLCPGWRHRRKKRPTFSRKPNSMSSNHAFSTSATRETLY
     FYMLTNALFYVSSAINPIL-YNLV
                                GKRFRKKSREVYOAICRKGGCMGESVOMENSMGTLRTSISVDRQIHKLQDWAGNKQ
41
     VTQISSYVAYSNSCLNPLV-YVIV
                                SOKRFAAFRKLCNCKOKPTEKAANYSVALNYSVIKESDRFSTELEDITVTDTYVSTTKVSFDDTCLASEN
     -LLKCRICIYLNSAINPVI-YNLM
42
                                NHRFRSGFRLAFRCCPWYTPTKEDKLELTPTTSLSTRVNRCHTKETLFMAGDTAPSEATSGEAGRPQDGSG-(17)
     VYLALFWLAMSSTMYNPII-YCCL
43
                                NDRFRLGFKHAFRCCPFISAGDYEGLEMKSTRYLQT-QSSVYKVSRLEITISTVVGAHEEEPEEGPKATPS-(29)
     VYLASMWLAMSSTMYNPII-YCCL
44
                                NKRFRAGFKRAFRWCPFIQVSSYDELELKTTRFHPTRQSSLYTVSRMESVTVLFDPNDGDPTKSSRKKRAV- (34)
     VYLASFWLAMSSTMYNPII-YCCL
45
                                GKKFKKYFLQLLKYIPPKAKSHSNLSTKMSTLSYRPSEQGNSSTKKPAPCIEVE
     AMPITICLAYFOONLNPLF-YGFL
46
                                GSSKKKRFKESLKVVLTRAFKDEMQPRRQKDNC-NTVTVETVV
     LHHISLLFSTINSSANPFI-YFFV
47
                                TKTFQRDFFLLLSKFGCCKRRADIYRRKDFSAYTSNCKNGFTGSNKPSQSTLKLSTLHCQGTALLDKTRYTEC
     VLLVLFYPI---NSCANPFL-YAIF
ILLVLFYPL---NSCANPFL-YAIF
48
                                TKAFQRDVFILLSKFGICKRQAQAYRGQRVPPKNSTDIQVQKVTHDMRQGALMMEDVVELIENSHLTPKKQ-(12)
49
                                TKNFRRDFFILLSKCGCYEMQAQIYRTETSSTVHNTHPRNGHCSSAPRVTSGSSTYILVPLSHLAQN
50
     ILLVLFHPI --- NSCANPFL-YAIF
                                NKQFRNCMLTTICCGKNPLGDDEASATVSKTETSQVAPA
     fmtipaffaksaaiynpvi–yimm
                                NROFRNCILOLFGKKVDDGSELSSASKTEVSSVSSVSPA
     MAALPAFFAKSATIYNPVI-YVFM
                                NROFRNCILOLFGKKVDDGSELSSASKTEVSSVSSVSPA
     MAALPAYFAKSATIYNPVI-YVFM
53
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54
     LVTIPSFFSKSACIYNPII-YCFM
      -TASVMYTVVTPMVNPFI-YSL-
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55
      -AAAVMYAVVTPMLNPFI-YSL-
                                RNSDMKAALRKVLAMRFPSKQ
56
      -AITVLNTIVTPVLNPFI-YTL-
                                RNKDVKEALRRTVKGK
57
                                RNKOVKRALERILLEGNCKVHHWTG
      --- SASVMYTVVTPMLNPFI -YSL-
58
59
      --- VMAMMYTVVTPMLNPFI -YSL-
                                RNRDMKRALIRVICSMKITL
                                RNODVKRALRRTLHLAQDQEANTNKGSKIG
60
      --LVSVLYAVIVPLFNPII-YCL-
      --- AMAMMYTVVTPLMNPFI-YSL-
                                RNRDMKQALIRVTCSKKISLPW
61
       -VMSLMYTMVTPMLNPFI-YSL-
                                RNRDIKDALEKIMCKKQIPSFL
62
                                RNRDMKRALIRVICTKKISL
       -AMAMMYTVVTPMLNPFI -YSL-
63
                                RNRDMKEALI RVLCKKKI TFCL
      --vmammytvvtpmlnpfi-Ýsl-
                                skdlrhafrsmfpscegtaqpldnsmgdsdclhkhanntasmhraaescikstvkiakvtmsvstdtsaeal
     VFAFCSMICLLNSTVNPII-YALR
65
                                NENFRVELKALLSMCQRPPKPEDRLPSPVPSFRVAWTEKSHGRRAPLPNHHLPSSQIQSGKTDLSSVEPVVAMS
     LYFAFHWFAMSSTCYNPFI-YCWL
     LFLICHLTAMISTCVNPIF-YGFL
                                NKNFORDLOFFFNFCDFRSRDGRTTRL
67
                                NKEMRRAFIRIMSCCKCPSGDSAGKFKRPIIAGMEFSRSKSDNSSHPQKDEGDNPETIMSSGNVNSSS
     AEYFLV-LAVLNSGTNPII-YTLT
68
                                KKFKETYFVI LRACRLCQTSDSLDSNLEQTTE
     AMCLGILLSHANSMINPIV-YACK
69
                                SDNFKRSFORI LCLSWMDNAAEEPVDYYATALKSRAYSVEDFQPENLESGGVFRNGTCASRI STL
     VSQLSVILGYANSCANPIL-YGFL
70
     PEYVIDLCICINSSAKPIV-YFLA
                                GRDKSQRLWEPLRVVFQRALRDGAEPGDAASSTPNTVTMEMQCPSGNAS
71
     IGTLARVVPHLHCLINPIL-YALL
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72
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     ALILTESIAFCHCCLNPLL-YVFV
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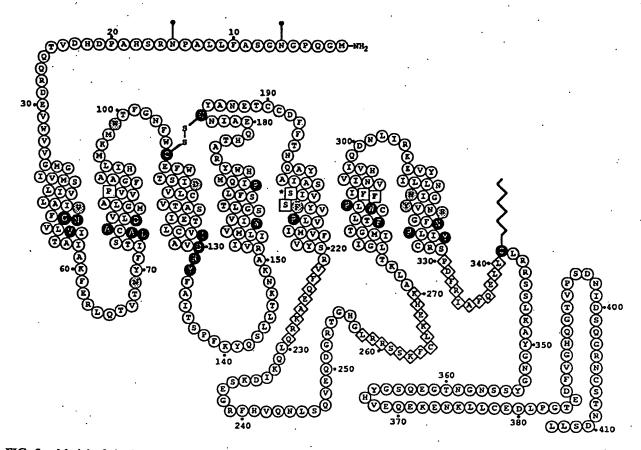


FIG. 3. Model of the human β_2 -adrenergic receptor. Amino acid residues in black are conserved in nearly all of the GPRs. Stippled residues are conserved in cationic amine receptors. Boxed residues are conserved in all catecholamine receptors. The asterisk denotes the serine conserved in the serotonin receptors. Residues in diamonds are residues believed to be involved in G-protein coupling. Glycosylated asparagines (N-6 and N-15) within the amino terminus are indicated. Cys³⁴¹ of the carboxyl terminus is known to be palmitoylated. Protein kinase A phosphorylation sites are indicated by arrowheads.

LIGAND BINDING DOMAINS

Our understanding of the structure of the binding site of the GPRs and of which residues actually interact with agonists and antagonists is rapidly evolving. The ligand binding sites of rhodopsin and of the adrenergic and muscarinic receptors have been partly delineated through biochemical and molecular biological approaches (for review, see Applebury and Hargrave, 1986; O'Dowd et al., 1989b; Strader et al., 1989b; Venter et al., 1989; Hulme et al., 1990). For most of the GPRs, with the possible exception of the glycoprotein hormone receptors, the ligand binding pocket appears to be created by the membrane-spanning regions.

As none of the GPRs have yet been crystallized, modeling of the three-dimensional array of the helices is based on the structure of bacteriorhodopsin, which has recently been resolved at high resolution (Henderson et al., 1990). The transmembrane domains appear to form a hydrophilic pocket for ligand binding surrounded by hydrophobic residues (Strader et al., 1989b; Venter et al., 1989; Hulme et

al., 1990). The putative arrangement of the residues around the ligand binding site have been analyzed through helical wheel modeling. The α -helices contains 3.6 residues per helical turn. When the assortment of residues around the helix is predicted for the muscarinic receptors (Hulme et al., 1990), adrenergic receptors (Strader et al., 1989b; Venter et al., 1989), and many other GPRs (Donnelly et al., 1989), the domains contain a predominance of hydrophobic residues on one side and hydrophilic on the other. The hydrophilic side of each helix is postulated to face inwards and form the polar ligand binding site. Recently, computer-generated models for ligand-receptor interactions have been developed (Findlay and Eliopoulos, 1990; Henderson et al., 1990; Dahl et al., 1990; Hibert et al., 1991).

The presence of a ligand binding pocket for the chromophore retinal deep within the transmembrane α -helices of rhodopsin was suggested by cross-linking and fluorescent energy transfer studies (Hargrave et al., 1982; Thomas and Stryer, 1982). Retinal forms a Shiff base linkage with Lys²⁹⁶ in TM 7 (Thomas and Stryer, 1982). The Glu¹¹³ of

rhodopsin in TM 3 has been proposed as a counterion that interacts with the protonated Shiff base retinal, although mutagenesis studies have been inconclusive (Sakmar et al., 1989; Zhukovsky and Oprian, 1989).

A variety of approaches support the existence of a similar intrahelical binding site in the cationic amine receptors. The ligand binding site of the adrenergic receptors has been investigated by photoaffinity labeling (Bar-Sinai et al., 1986; Dohlman et al., 1988), fluorescence emission spectroscopy (Tota and Strader, 1990), deletion mutants (Dixon et al., 1987a,b), site-directed mutagenesis (Chung et al., 1988; Dixon et al., 1988; Strader et al., 1988; Fraser, 1989; Strader et al., 1989a,b; Wang et al., 1991), and receptor chimeras (Kobilka et al., 1988). As was the case for the visual pigments, the transmembrane domains are necessary for ligand binding and confer ligand specificity, while the hydrophilic extracellular and intracellular domains are not directly involved in ligand binding (Dixon et al., 1981a,b). In both the α - and β -adrenergic receptors (Strader et al., 1988; Wang et al., 1991) as well as the m. muscarinic receptor (Fraser et al., 1989), site-directed mutagenesis has demonstrated that the TM 3 aspartate (Asp¹¹³ in the β-adrenergic receptor; see Fig. 3) is critical for wild-type agonist and antagonist binding. The cationic amines, which include epinephrine, norepinephrine, dopamine, serotonin, and acetylcholine, all contain a positively charged amine head group which most likely interacts with the conserved TM 3 aspartate found in these receptors.

Mutagenesis studies have suggested that particular residues conserved within receptor subclasses can contribute to agonist specificity. Two conserved serines in TM 5 (Ser²⁰⁴ and Ser²⁰⁷ in the β-adrenergic receptor) have been implicated in forming hydrogen bonds with the meta- and para-hydroxyl groups of adrenergic agonists. Replacement of either serine by alanine reduces agonist binding to the same degree as removing the corresponding hydroxyl group from the ligand (Strader et al., 1989a). Recent mutagenesis studies of the \alpha_2-adrenergic receptor suggest that Ser²⁰⁴ (corresponding in position to Ser²⁰⁷ of the β-adrenergic receptor) binds in an analogous fashion to the para-hydroxyl group of adrenergic ligands (Wang et al., 1991). Two corresponding serine residues are found in TM 5 in all the dopamine receptors and a single conserved serine residue in TM 5 of the serotonin receptors (Fig. 2). The similarity of ligand structure and receptor sequence suggests that these TM 5 serines may also hydrogen bond with the aromatic hydroxyl groups of their respective agonists. This hypothesis is supported by computer modeling of the ligand-receptor interaction (Hibert et al., 1991). The muscarinic receptors all contain a conserved asparagine in TM 6, not found in any other receptor subclass, which has been proposed to interact with the ester group of acetylcholine (Hibert et al., 1991). Conserved TM 6 and/or TM 7 aromatic residues (e.g., Phe²⁹⁰ and Tyr³²⁶ in the β -adrenergic receptor) may interact with the aryl ring of serotonergic and adrenergic ligands (Dixon et al., 1988; Hibert et al., 1991).

The ligands for the glycoprotein hormone receptors, thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), and lutenizing hormone/chorionic

gonadotropin (LH/CG), are much larger than the ligands for the other GPRs. Presumably because of the large size of the ligands, this receptor subclass has evolved a distinct structure containing an extremely long first cytoplasmic domain encompassing the high-affinity hormone binding site. This glycosylated extracellular domain is rich in cysteine residues that may form disulfide bridges and help maintain the three-dimensional structure of the proteins (Sprengel et al., 1990). The large amino-terminal extracellular domain of these receptors contains multiple leucinerich repeats that identify these GPRs as members of a second gene family, that of the leucine-rich glycoprotein family (Takahashi et al., 1985; Krusius and Ruoslahti, 1986). The extracellular location of a hormone binding site is supported by chimera studies (Moyle et al., 1991; Nagayama et al., 1991b) and, in the case of the LH/CG receptor, by the secretion of a soluble hormone binding protein generated by alternative splicing which encompasses only the amino terminus (Loosfelt et al., 1989; Tsai-Morris et al., 1990). Short regions of the amino terminus of TSH and LH/CG are necessary for high-affinity hormone binding (Wadsworth et al., 1990; Nagayama et al., 1991a,b). In contrast, \(\beta\)-adrenergic receptor ligand binding is not dependent on the amino-terminal extracellular domain (Dixon et al., 1987b).

Recently the binding and activation of an LH/CG receptor construct in which virtually the entire extracellular amino terminus has been deleted was investigated (Ji and Ji, 1991b). The finding that CG can bind to the seven transmembrane components of the receptor, albeit with lower affinity, in the absence of the extracellular amino terminus suggests that this receptor may contain both a high-affinity binding site extracellularly and a low-affinity site within the transmembrane domains. CG binding to this low-affinity receptor mutant was capable of stimulating cAMP production. Possibly the high-affinity extracellular binding site serves to capture the hormone and present it to the intramembranous binding pocket for signal transduction.

INTRACELLULAR COUPLING

The GPRs are coupled by heterotrimeric G-proteins to various intracellular enzymes, ion channels, and transporters (Johnson and Dhanasekaran, 1989; Birnbaumer et al., 1990). The G-proteins, which associate with GPRs at the intracellular face of the plasma membrane, are composed of relatively invariant β - and γ -subunits and a variable α subunit $(\alpha_s, \alpha_i, \alpha_o)$ for which the G-protein is named $(G_s,$ G_i, G_o). By a process not yet understood, GPR agonist binding catalyzes the exchange of GTP for GDP on the α-subunit (G-protein "activation"), resulting in its dissociation and stimulation of one (or more) of the various signaltransducing enzymes and channels. The different G-protein a-subunits preferentially stimulate particular effectors. The specificity of signal transduction may be determined, therefore, by the specificity of G-protein coupling. Some GPR residues or regions which are necessary for efficient signal transduction can be postulated to interact with conserved G-protein motifs. In addition, certain short amino acid stretches of the receptors which are necessary for G-protein coupling also determine the specificity of the G-protein interactions.

Three types of studies investigating the relationship between receptor structure and G-protein affinity have been performed. Deletion and site-directed mutagenesis studies implicate receptor regions and amino acid residues that are necessary for efficient G-protein coupling. Synthetic peptide competition studies suggest which oligopeptide domains may directly interact with the G-proteins. Chimera experiments delineate the receptor regions that determine the specificity of G-protein coupling. Certain general principles arise from these multifaceted investigations. All of the intracellular domains are implicated in efficient G-protein coupling of various receptors. Short stretches of the membrane proximal regions of the third cytoplasmic loop and possibly the carboxyl terminus appear particularly critical in determining the specificity of G-protein coupling for many receptors.

Single residue mutations in the cytoplasmic loops of the β -adrenergic receptor reduce signal transduction (Dixon et al., 1988; O'Dowd et al., 1988). Site-directed mutagenesis of a conserved proline in the second intracellular loop to threonine, for example, caused no change in agonist binding but a ~35% reduction in adenylate cyclase stimulation (O'Dowd et al., 1988). Site-directed mutagenesis has identified particular charged residues in the membrane proximal regions of the second and third intracellular loops which contribute to efficient G-protein coupling. Mutation of the highly conserved aspartate adjacent to TM 3 in the second intracellular loop of the β -adrenergic receptor (Asp130 which is in the "DRY" sequence) gives rise to a receptor with high-affinity ligand binding but reduced or absent G-protein coupling (Dixon et al., 1988; Fraser et al., 1988). Similar results have been obtained for the muscarinic m_1 and the α_{2A} -adrenergic receptors (Fraser et al., 1988, 1989; Wang et al., 1991). The corresponding glutamate of rhodopsin is similarly implicated as interacting with transducin (Franke et al., 1990). Another residue needed for transducin activation by rhodopsin is the lysine located in the distal third intracellular loop, Lys246. Mutation of this lysine to leucine results in a complete loss of signal transduction (Franke et al., 1988). Mutation or deletion of histamine at the corresponding position in the β adrenergic receptor reduced, although did not abolish, adenylate cyclase stimulation (O'Dowd et al., 1988).

The TM 2 aspartate (Asp⁷⁹ of the β -adrenergic receptor, see Fig. 3), which is conserved in virtually all GPRs, is necessary for wild-type agonist binding and G-protein activation in many GPRs studied (Chung et al., 1988; Strader et al., 1988; Fraser et al., 1989; Wang et al., 1991; Ji and Ji, 1991a). In the α_2 -adrenergic and dopamine D₁ receptors, this aspartate is essential for modulation of receptor coupling by Na⁺ and H⁺, possibly due to allosteric modulation of receptor conformation (Horstman et al., 1990; Neve, 1991; Neve et al., 1991). Another transmembrane residue, the TM 6 cysteine found in most GPRs, has been implicated in β -adrenergic receptor signal transduction (Fraser, 1989).

Deletion studies of the β_2 -adrenergic receptor have indicated that the membrane proximal regions of the third cytoplasmic loop (residues 222-229 and 258-270, see Fig. 3) are necessary for signal transduction (Strader et al., 1987a; O'Dowd et al., 1988). In the α_1 -adrenergic receptor, deletion of seven amino acids of the third intracellular loop proximal to TM 7 caused a marked reduction in coupling to phospholipase C (Cotecchia et al., 1990).

Deletions of carboxy-terminal residues adjacent to TM 7 produce mutant rhodopsin or β_2 -adrenergic receptors with diminished ability to activate G-proteins (O'Dowd et al., 1988; Franke et al., 1990). Mutation of a palmitoylated carboxy-terminal Cys341 to glycine markedly reduced agonist stimulation of adenylate cyclase of the β2-adrenergic receptor (O'Dowd et al., 1989a). The palmitoylated cysteine is predicted to anchor the carboxyl terminus to the membrane, producing a fourth cytoplasmic loop (see Fig. 3). Membrane anchorage may optimally position carboxyterminal residues for G-protein interaction (Ovchinnikov et al., 1988; O'Dowd et al., 1989a). Regions of the carboxyl terminus and third cytoplasmic loop, adjacent to the transmembrane domains, may form clustered amphipathic α-helices (Strader et al., 1987a; Higashijima et al., 1988; Strader et al., 1989b; Palm et al., 1990). These helices, along with charged intracellular residues of the second and third intracellular loops (i.e., DRY), may cooperatively interact to efficiently bind and activate G-proteins.

The activation of G-proteins by amphipathic α -helices is supported by experiments in which the G proteins G_i and G_0 have been directly activated by mastoparan and other small peptides which form amphipathic α -helices at the inner surface of the cytoplasmic membrane (Higashijima et al., 1988, 1990). Furthermore, direct activation of G_s has been demonstrated for synthetic peptides representing the third intracellular loop sequences adjacent to TM 5 and TM 6 of the β_2 -adrenergic receptor (Cheung et al., 1991), and by a peptide representing the intracellular third loop sequence proximal to TM 6 of the avian β -adrenergic receptor (Palm et al., 1989; Munch et al., 1991).

Peptide competition experiments, in which short synthetic peptides competitively bind to G-proteins but do not activate them, have been useful in mapping GPR regions that are likely to contact the G-proteins. Receptor uncoupling following mutagenesis or deletion of receptor segments may be due either to loss of G-protein contacts or to altered tertiary structure of the receptors. Competition studies have been invaluable, therefore, in confirming that the loss of signal transduction observed in deletion and mutagenesis studies involves residues that directly bind the G-proteins. The regions of various receptors implicated by peptide competition studies include the membrane proximal regions of all three cytoplasmic loops and the carboxyl terminus of the avian β -adrenergic receptor (Palm et al., 1989; Munch et al., 1991), the second intracellular loop and the carboxyl terminus of the third intracellular loop of the α_{2A} -adrenergic receptor (Dalman and Neubig, 1991). and the second and third intracellular loops and amino-terminal region of the carboxyl terminus of rhodopsin (Konig et al., 1989).

Chimera experiments involving hybrid α₂/β₂-adrenergic

receptors suggested that the third cytoplasmic loop may underlie coupling specificity of the adrenergic receptors (Kobilka et al., 1988). The β_1 -receptor is positively coupled to adenylate cyclase through G_s, whereas the α_2 -receptor is negatively coupled to this enzyme through Gi. \$2-Adrenergic receptors were generated in which the third cytoplasmic loop was replaced by the third cytoplasmic loop of the a2adrenergic receptor. Activation of this chimeric receptor, which still has β_2 pharmacology, caused inhibition instead of stimulation of adenylate cyclase (Kobilka et al., 1988). Similar results have been obtained for other cationic amine receptor hybrids. The dopamine D₂ receptor is negatively coupled to adenylate cyclase, whereas the β2-adrenergic receptor is stimulatory to adenylate cyclase. Substitution of the third cytoplasmic loop of the phospholipase-coupled muscarinic m, receptor into the dopamine D2 receptor and of the same region of the phospholipase-linked a1-adrenergic into the \$2-adrenergic receptor caused the resultant chimeras to hydrolyze phosphatidylinositol and mobilize calcium (Cotecchia et al., 1990; England et al., 1991).

The receptor region of the third cytoplasmic loop most important in determining the specificity of signal transduction may differ between the muscarinic and adrenergic receptors. The signal transduction of α_2/β_2 -adrenergic receptor chimeras, in which short segments of the membrane proximal regions of the third intracellular loops and of the carboxyl terminus have been exchanged, indicated that the segment of the third intracellular loop adjacent to TM 6 is most important in adrenergic receptor coupling specificity (Liggett et al., 1991). Substitution of multiple segments suggested that all of these domains may coordinately contribute to G-protein coupling (Liggett et al., 1991). By contrast, interchange of seven amino acids from the third intracellular loop adjacent to TM 5 was sufficient to change the coupling specificity of a muscarinic m1/m2 chimera (Kubo et al., 1988). In another series of experiments, substitution of nine amino acids from the amino terminus of this region of the third cytoplasmic loop of the muscarinic m, receptor into the m, receptor conferred a pattern of calcium release characteristic of m, receptor activation (Lechleiter et al., 1991).

Phosphorylation of cytoplasmic residues has been identified as an important mechanism for the regulation of G-protein coupling of some GPRs. The third cytoplasmic loop and carboxyl terminus are rich in serine and threonine residues that are potential phosphorylation sites. After activation, both rhodopsin and the β_2 -adrenergic receptors are desensitized through the action of receptor kinases. The photoactivated form of rhodopsin is phosphorylated in the carboxyl terminus by a specific rhodopsin kinase (Hargrave et al., 1982). This phosphorylation allows binding of the protein arrestin, which interferes with G-protein coupling to the opsin. A similar mechanism has been identified for the β_2 -adrenergic receptor, in which β -adrenergic receptor kinase (BARK) phosphorylates the carboxyl terminus of the receptor. This leads to binding of a β -arrestin and functional uncoupling of the receptor. BARK can also phosphorylate the third cytoplasmic loops of agonist stimulated m₄ muscarinic and α₂-adrenergic receptors, as well as the carboxyl terminus of photoactivated rhodopsin

(Benovic et al., 1986, 1987a; Kwatra et al., 1989). Many receptors contain cytoplasmic consensus sequences for protein kinase A phosphorylation. In the case of the β -adrenergic receptors these sites play a role in receptor desensitization (Clark et al., 1989). The TSH receptor, which does not contain consensus sequences for protein kinase A phosphorylation, does not demonstrate agonist-induced desensitization (Takasu et al., 1978).

Structure/function modeling of the mechanism of Gprotein activation by GPRs must also account for the recent identification of G-protein coupled receptors which are not members of the GPR gene family and of peptides that are capable of directly activating G-proteins. The secretin receptor, while distinct in sequence, is predicted to exhibit a seven-transmembrane domain structure (Ishihara et al., 1991). Although the metabotropic glutamate receptor also manifests seven closely spaced hydrophobic domains, the hydrophobicity profile predicts an additional potential membrane spanning domain distant from the other seven (Masu et al., 1991). The activation of heterotrimeric G-proteins has been implicated in the signal transduction of several membrane receptor tyrosine kinases. These related receptors, which bear no overall structural or sequence resemblance to the GPR family, include the insulin receptor, the insulin-like growth factor-II receptor, the epidermal growth factor receptor, and the colony stimulating factor-1 receptor encoded by the c-fms proto-oncogene (Imamura and Kufe, 1988; Nishimoto et al., 1989; Luttrel et al., 1990; Liang and Garrison, 1991). A 14amino-acid segment of the insulin-like growth factor-II receptor, which bears striking resemblance in its charge distribution to the amphipathic protein mastoparan and to the membrane proximal regions of the third cytoplasmic loop of the GPRs, specifically activates the heterotrimeric G-protein, Gi (Okamoto et al., 1990; Nishimoto et al., 1991). The oncogenic activity of the v-fps protein, a cytosolic tyrosine kinase, may also involve activation of a heterotrimeric G-protein (Alexandropoulus et al., 1991). GAP-43 is a growth cone protein that activates Go- A decapeptide domain of GAP-43 which is homologous to the membrane proximal carboxyl terminus of many GPRs was found to be responsible for association with Go (Strittmater et al., 1990). Amphiphilic neuropeptides, including substance P, ACTH, and bradykinins, can also activate G-proteins in a receptor-independent fashion (for review see Mousli et al., 1990). Further delineation of the structural motifs that mediate G-protein coupling of these nonhomologous receptors and peptides would be expected to illuminate the mechanisms of receptor/G-protein interaction in general.

GENE STRUCTURE AND EVOLUTION

Molecular cloning has revealed that a panoply of receptor subtypes exist for most of the classical neurotransmitters. As the basic transmitters developed very early phylogenetically (see Walker and Holden-Dye, 1989), the subsequent evolution of multiple receptor subtypes served the need for greater signaling specificity of progressively

more complex nervous systems. Nucleotide sequence analysis and analysis of gene structure may elucidate the time frame and mechanisms of subfamily and subtype evolution.

The remarkable conservation of the transmembrane domains of the GPR family proteins suggests that these genes may have evolved from a common precursor. A phylogenetic tree of the GPR family, generated by nucleotide sequence comparison, suggests that the opsins diverged from the catecholamines between 1 and 1.5 billion years ago (Yokoyama et al., 1989). The age of the GPR gene family is independently suggested to be greater than 1 billion years by the isolation of a Dictyostelium chemoattractant receptor with structural and sequence homology with the GPRs (Klein et al., 1988). While several seven-transmembrane yeast pheromone receptors have been identified (Hagen et al., 1986; Marsh and Herskowitz, 1988), these proteins have little amino acid sequence homology with the GPRs. The evolutionary relationship, if any, between these yeast receptors, the seven-transmembrane bacteriorhodopsin, and the GPR superfamily remains to be determined.

Many of the GPR genes characterized to date are intronless. Several GPR genes, however, have introns within their coding regions. These include the opsins (Nathans and Hogness, 1984; Nathans et al., 1986), the dopamine D2, D3, and D4 receptors (Grandy et al., 1989; Sokoloff et al., 1990; Gandelman et al., 1991; Van-Tol et al., 1991), the substance P receptor (Hershey et al., 1991), the substance K receptor (Gerard et al., 1990), the lutenizing hormone receptor (Frazier et al., 1990; Tsai-Morris et al., 1990), and a Drosophila muscarinic receptor gene (Shapiro et al., 1989). Introns have not been found within the coding regions of mammalian muscarinic receptor genes isolated to date. For the GPR genes with introns, the locations of introns near or within the seven transmembrane domains, are illustrated in Fig. 4. Introns tend to be positioned between TM domains.

Two mechanisms of gene evolution, gene duplication and retroposition, both appear to have played a role in generating the complex multiplicity of the GPR family. Genes containing introns, like those for the dopamine D2, D₃, and D₄ receptors, most likely evolved from each other by gene duplication (Ohno, 1970). This is supported by the relative preservation of intron location among these receptors (see Fig. 4). There is also the preservation of an intron site (adjacent to the region encoding TM 3), between the dopamine and tachykinin receptor genes and of a different intron site (adjacent to the region encoding TM 7) between the tachykinin receptor and opsin genes, suggesting that gene duplication of a common precursor may have played a role in the evolution of these receptors. Most of the receptor genes are intronless, raising the possibility that one or more of these arose through reverse transcription of mRNA and incorporation into the genome (Brosius, 1991), an event referred to as retroposition. Gene duplication may have further amplified the number of these intronless. genes.

Another potential mechanism for generating functionally distinct receptors is alternative processing of RNA primary transcript. Alternative splicing of a free-standing exon of the dopamine D2 receptor gene gives rise to two receptor isoforms which differ in the incorporation or absence of a 29-amino-acid segment of the third cytoplasmic loop (Grandy et al., 1989). Although the functional difference between the two isoforms remains to be elucidated, their biological importance is suggested by the preservation of the alternative splice site through at least 80 million years of evolution, from mouse to man (Montmayeur et al., 1991). Alternative splicing also gives rise to multiple forms of the dopamine D₃ receptor mRNA (Giros et al., 1991; Snyder et al., 1991) and of the LH/CG receptor. An alternative mRNA splice variant of the LH/CG receptor encodes a secreted LH binding protein lacking the transmembrane regions (Loosfelt et al., 1989; Tsai-Morris et al., 1990).

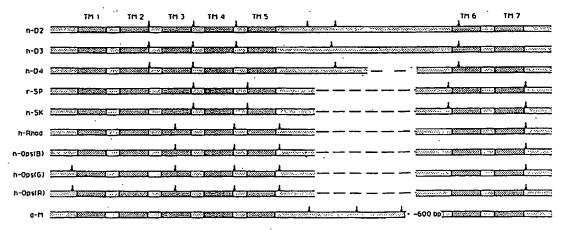


FIG. 4. Schematic representation of GPR genes that have introns within the protein coding region. Abbreviations: h-D₂, human dopamine D₂ receptor; r-D₃, rat dopamine D₃ receptor; h-D₄, human dopamine D₄ receptor; r-SP, rat substance P receptor; h-SK, human substance K receptor; h-Rhod, human rhodopsin; h-ops(B), human blue opsin; h-ops(G), human green opsin; h-ops(R), human red opsin; d-M, *Drosophila* muscarinic receptor. The locations of introns are indicated by arrows.

Convergent evolution is also evident in G-protein coupled receptors. The examples of secretin and the metabotropic glutamate receptor in which apparently unrelated genes have evolved similar seven-transmembrane structures and G-protein coupling have already been discussed. Comparison of the nucleotide sequence for the red and green visual pigment genes between fish and human indicate that the red pigments evolved independently from the green pigment through identical amino acid substitutions (Yokoyama and Yokoyama, 1990).

SUMMARY

The identification of new GPR genes and the elucidation of their binding and G-protein coupling mechanisms will undoubtedly continue to accelerate. In particular, the binding site and coupling domains of the non-glycoprotein hormone peptide receptors have not yet been investigated and their study will help illuminate the binding and coupling characteristics in this family. Although striking progress has been made in delineating the ligand binding site of rhodopsin and the cationic amine GPRs and the structural motifs contributing to G-protein recognition, the actual molecular events which transmit ligand binding into activation of G-protein remain to be elucidated. More complete understanding of the GPR's three-dimensional structure, pharmacology, physiology, and anatomy will ultimately have a tremendous impact on our understanding of biology and on the development of pharmaceuticals.

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